EXPRESSION AND PROGNOSTIC SIGNIFICANCE OF E CADHERIN, EGFR, P53 AND HER-2/NEU IN GASTRIC CARCINOMA

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CERTIFICATE

This is to certify that this Dissertation entitled "**EXPRESSION AND PROGNOSTIC SIGNIFICANCE OF E CADHERIN, EGFR, P53 AND HER-2/NEU IN GASTRIC CARCINOMA**" is the bonafide original work of **Dr. S. THARAGESWARI**, in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R Medical University to be held in April 2013.

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DECLARATION

I Dr. S. Tharageswari, solemnly declare that the dissertation titled **"EXPRESSION AND PROGNOSTIC SIGNIFICANCE OF E CADHERIN, EGFR, P53 AND HER-2/NEU IN GASTRIC CARCINOMA"** is the bonafide work done by me at Institute of Pathology, Madras Medical College under the expert guidance and supervision of Prof. Dr. P. Karkuzhali, M.D., Director and HOD of Institute of Pathology and Electron Microscopy, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr.M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place: Chennai
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ABBREVIATIONS

EGFR- Epidermal Growth Factor Receptor

HNPCC- Hereditary Non polyposis Colorectal Carcinoma

WHO- World Health Organization

MLH - 1 : MutL Homolog – 1gene

hPMS : human Protein Homolog gene

hMSH : human MutS Homolog gene

PGP 9.5 : Protein Gene Product 9.5

AJCC- American Joint Committee on Cancer

cDNA- Complementary Deoxyribo Nucleic Acid

PCR- Polymerase Chain Reaction

FISH- Fluorescent In Situ Hybridisation

IHC- Immunohistochemisrty

HRP- Horse Radish Peroxidase

GIST- Gastro Intestinal Stromal Tumour

CI - Confidence Interval

RGGGH- Rajiv Gandhi Government General Hospital

CONTENTS

INTRODUCTION

Stomach cancer is the fourth most common cancer worldwide [1]. It is a disease with a high death rate (~700,000 per year) making it the second most common cause of cancer death worldwide after lung cancer[2]. It is more common in men than in women with a ratio of 1.6:1[1, 3]. It is a disease of middle aged and elderly with a peak incidence at around 65 years [1]. Although a steady decline in the incidence of gastric carcinoma has been noted worldwide, the absolute number of new cases per year is increasing mainly because of the aging population. Decline in incidence rate is seen in the western countries [4]. In Asia it is still one of the most common malignancies accounting for 18% of all malignancies. In countries like Japan and Korea it accounts for 56% of malignancies [5].

More than 50% of cases present in advanced unresectable stages making cure impossible. The overall 5 year survival rate is 28% irrespective of the stage at which the patient presents. The morphological and histological types of gastric carcinomas are variable and they may not correlate well with the prognosis. The prognosis is mainly dependant on the clinical stage of the disease. But even within a specific stage, there is variability in prognosis from patient to patient. There has been a constant search for specific biological markers to identify the subgroups of patients with more aggressive course of the disease [6]. E Cadherin, EGFR, p53 and

HER-2/Neu have been proposed as potential tool for the evaluation of prognosis of gastric cancer [7, 8, 9, 10].

E Cadherin plays a crucial role in cell- cell adhesion in epithelial tissues. The decreased E Cadherin expression correlates with dedifferentiation, infiltrative tumour growth, distant metastasis, and poor survival for patients with certain carcinomas [7].

Epidermal Growth Factor Receptor (EGFR) is a transmembrane tyrosine kinase receptor, and is one of the members of EGFR family of receptors. This receptor activation causes stimulation of the downstream signaling pathway that regulates cell proliferation, migration, adhesion, differentiation and survival. Gene amplification and / or protein over expression of EGFR have been observed in a variety of solid tumours [8].

p53 is a tumour suppressor gene which negatively regulates cell cycle. A loss of function mutation in p53 gene results in enhanced proliferative activity and tumour progression. In contrast to the normal p53 protein, the mutated p53 protein has an increased half-life and hence accumulates within the cell nucleus. This can be detected immunohistochemically using monoclonal antibodies [9].

HER-2/Neu, also known as c-Erb-2, encodes a transmembrane tyrosine kinase receptor; homologous to epidermal growth factor receptor

.The protein encoded by this gene is suggested to be a growth factor receptor involved in the growth and progression of malignant cells [10].

Despite many studies that have been conducted in gastric carcinoma worldwide for the expression of E Cadherin, EGFR, p53 and HER-2/Neu and for their prognostic importance, the results are still contradictory. Some found a statistically significant association of these markers with prognosis and survival, while others found no such association. In a related development, targeted therapy against EGFR and HER-2/Neu has attracted much attention recently. Hence accurate evaluation of expression of EGFR and HER-2/Neu proteins might help to identify eligible candidates for new targeted therapy.

In this study, an attempt is made to study the expression of these four markers in gastric carcinoma immunohistochemically and compare it with various clinicopathological parameters and to study their prognostic significance.

AIMS AND OBJECTIVES

1. To study the incidence and distribution of gastric carcinoma in patients who attended Rajiv Gandhi Government General Hospital, during January to December 2011.
2. To study the various clinicopathological factors of gastric carcinoma including age of incidence, sex preponderance, tumour location, tumour size, gross appearance, depth of infiltration, lymphovascular invasion and perineural invasion.
3. To determine the immunohistochemical expression of E Cadherin, EGFR, p53 and HER-2/Neu in Gastric carcinoma.
4. To study the association of E Cadherin, EGFR, p53 and HER-2/Neu in Gastric carcinoma with known prognostic factors like age, sex, tumour size, histological type, grade, depth of infiltration, lymphovascular invasion, lymphocytic response.
5. To study the prognostic significance of E Cadherin, EGFR, p53 and HER-2/Neu in Gastric carcinoma and its association with survival.

REVIEW OF LITERATURE

Gastric carcinoma is a malignant neoplasm arising from the glandular epithelial lining of stomach mucosa. The benign and malignant gastric ulcers were first described by J.Cruveilhier, in 1835 [11]. The first successful operation, a subtotal resection with gastroduodenal anastomosis, was performed on 1881, by Theodor Billroth in Vienna. The specimen showed Gastric cancer involving the distal part of the stomach with all regional nodal involvement [13]. The patient died of recurrence after 4 months of surgery. Sixteen years later, in 1897, first successful total gastrectomy was performed by Karl Schlatter in Zurich [12].

Subsequently during the late nineteenth and twentieth centuries and now, in the early twenty-first century, millions of patients have been recognized to be affected by gastric cancer and submitted to surgery.

Epidemiology:

The incidence of gastric cancer between countries shows considerable variation. Over 934,000 cases are diagnosed each year [1]. Japan and Korea have the highest gastric cancer rates in the world [14, 15]. In Japan, the age-standardized incidence rates are 69.2 per 100,000 in men and 28.6 per 100,000 in women [16]. In India, there is a wide variation in the incidence of gastric carcinoma. According to the study conducted

by the National Cancer Registry Programme of India in 2001, the number of new gastric cancer cases were estimated to be approximately 35,675 (23,785 in men; 11,890 in women)[17]. The incidence rate of gastric cancer was four times higher in Southern India compared with Northern India[18]. Among the six registries in Southern India, the highest incidence in both sexes was reported from Chennai. The age-standardized incidence rates in Chennai are 13.6 per 100,000 in men and 6.5 per 100,000 in women[17]. The rates in rural population were much lower than those in the urban population. Early gastric cancer has a higher five year survival rate (up to 95%) than those of advanced gastric cancer (10% -20%)[19].

Clinical Presentation:

Early gastric cancers are usually asymptomatic. Advanced gastric cancers have non-specific symptoms like dysphagia, vomiting or regurgitation, epigastric distress or pain, hematemesis, melena, anorexia, weight loss and fatigue. Usually proximal gastric cancer causes dysphagia and distal gastric cancer causes gastric outlet obstruction.

Etiopathogenesis:

Gastric carcinogenesis is a multistep and multifactorial process. Progression from normal gastric mucosa through chronic gastritis, atrophic gastritis with or without intestinal metaplasia to dysphagia and to carcinoma is seen in many cases[20]. The risk factors associated with

gastric carcinoma include chronic intestinal metaplasia and atrophic gastritis, Helicobacter pylori infection, dietary factors like food rich in salt & nitrates (dried and salted fish) and low in micronutrients/ antioxidants (vitamin C), smoking, pernicious anaemia, previous history of gastric surgery having gastric stumps, Menetrier's disease and peptic ulcer disease[22]. First-degree relatives of affected patients are at three times risk for developing the disease when compared to the general population.

Helicobacter pylori infection:

Three independent prospective cohort studies showed epidemiological evidence of strong association with H Pyroli infection. Patients with gastric carcinoma had anti H Pyroli antibodies detectable in their serum stored 10 or more years before the diagnosis of cancer.[23,24,25]. At the pathogenesis level H Pylori has been shown to induce phenotypic changes such as mucosal atrophy, intestinal metaplasia and dysplasia, leading to the development of adenocarcinoma.[26] The development of severe gastritis with atrophy and intestinal metaplasia is particularly associated with infection by CagA-positive strains of the bacillus[27,28] . The various mechanisms proposed are increased epithelial cell proliferation with a resultant increased risk of mutations[29], bacterial overgrowth with increased potential to generate intraluminal

carcinogens[30], increased free radicals[31] and reduced gastric antioxidant levels[32].

Diet:

Most consistent association with dietary factor is observed in intestinal type of gastric carcinoma. Fresh fruits and vegetables lower the risk due to the antioxidant actions of ascorbic acid, carotenoids, folates, tocopherols[33,34]. Salt intake, smoked foods, pickled vegetables, chilli pepper are found to be associated with high risk[35,36,37].

Bile reflux:

The relative risk of adenocarcinoma is on a rise in the gastric stump of patients who have undergone previous gastric surgery 5 to 10 years ago. The risk is especially high in Bilroth II operation which increases the risk of bile reflux[38,39].

Host factors:

There is evidence that germline truncating mutations in the gene for E Cadherin, a calcium-dependent cell adhesion protein, are responsible for a rare autosomal dominant inherited form of gastric carcinoma in young persons. This condition is characterized by multiple tumours of diffuse or signet ring cell histological types that do not arise in a background of intestinal metaplasia[40]. Affected family members can be identified by mutation-specific genetic testing and offered prophylactic

gastrectomy[41]. Patients with hereditary non-polyposis colorectal cancer (HNPCC), which results from germline mutation of one of the DNA mismatch repair genes hMLH1, hMSH2, hMSH6, hPMS1 and hPMS2 also have an increased frequency of gastric cancers[42]. Peutz– Jegher's syndrome also shows an increased risk of gastric cancer[43].

Anatomical Distribution of Gastric Carcinoma:

Gastric carcinoma that arises from the distal part (antro-pyloric region and lesser curvature) is more common than those arising from the proximal part (body and cardia). In the past decade there is a considerable increase in the incidence of cancer of the cardia and this is being attributable to widespread use of endoscopy and improvements in diagnostic methods. Proximal carcinomas are more aggressive than the distal gastric carcinomas.

Early gastric cancer:

Gastric carcinoma confined to mucosa and submucosa, with or without lymph nodal metastasis is defined as early gastric cancer. The term 'early' implies the disease is potentially curable and not the early stage in the genesis of the tumour[55]. Usually they are asymptomatic. Most frequently reported symptoms are epigastric pain and dyspepsia, occurring within few months before diagnosis[45].

Most of these lesions are small (2 to 5cms), typically located in lesser curvature around antropyloric region. Multiple primary sites are seen in 3 to 13% of cases and are associated with bad prognosis[46].

Based on endoscopic appearance, three types of Early gastric carcinomas are identified:

Type I : protruding

Type II : Superficial- II A- elevated

- II B- flat

- II C- depressed

Type III :Excavating

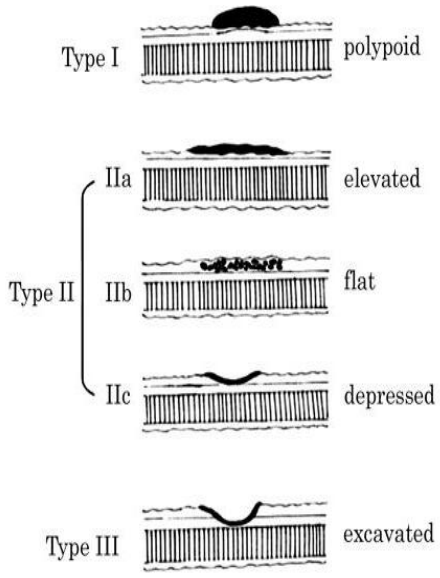


Figure 1: Classification of early gastric cancer based on endoscopic appearance

Type II is more frequent (80%) of which type II C is the most common one.

Microscopic variants include “minute gastric cancer”(less than 5 mm)[46] and “Superficial spreading type” which shows serpiginous ulcers with neoplastic cells that spread laterally over large surface area of mucosa.

Elevated lesions are usually well differentiated and intestinal type, flat lesions and depressed ones are usually poorly differentiated, intestinal or diffuse types.[47-49] Lymph node involvement occurs in 10 – 20% of all cases[46,47,50]. 63% cumulative risk of progression to advanced gastric cancer is seen in untreated cases[51]. 5 year survival rate in surgically resected cases is more than 90%[52,53]. Recurrence rate is 5 to 15%[54].

Advanced Gastric Carcinoma:

When the tumour invades beyond submucosa of stomach wall, it is called as advanced gastric carcinoma. It implies that resection and cure of the tumour is difficult and does not indicate that the tumour is of higher stage.

Based on gross appearance, Dr.R.Borrman gave the following classification.

Type I – Polypoid / Nodular

Type II – Ulcerative, localized / Fungating

Type III – Ulcerative, infiltrative

Type IV – Diffusely infiltrative (linitis plastica)

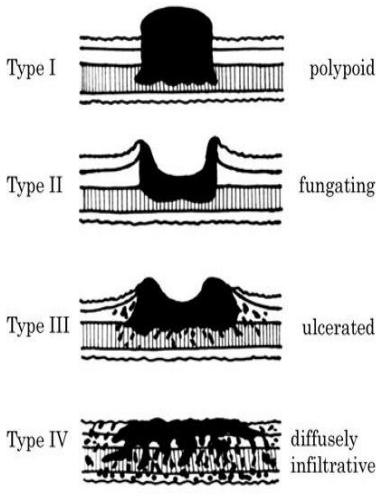


Figure 2: Borrman's Classification based on gross appearance

Ulcerated tumours occur most frequently in the antrum, on the lesser curve. The ulcers are large with irregular margins, raised rolled out edges, necrotic shaggy base[(56)]. Polypoid, nodular and fungating tumours usually occur in the body of the stomach in the region of the greater curvature, or fundus. Infiltrative cancers spread superficially, producing plaque-like lesions. It is usually accompanied by thickening of the entire stomach wall producing the so-called linitis plastica (leather bottle) stomach. Many gastric carcinomas secrete considerable amounts of mucin and give the gelatinous appearance of colloid carcinomas.

Microscopic appearance:

Several classifications based on the histological picture exist for gastric carcinoma. A few of the commonly used ones are the following.

Lauren's classification: (1965)[57]

The histological classification of Lauren divides gastric adenocarcinoma into two main types - Intestinal and Diffuse. Tumours with approximately equal quantities of intestinal and diffuse components are called indeterminate/unclassified carcinomas. Also, carcinomas too undifferentiated to fit neatly into either category are placed in the indeterminate/ unclassified category.

Of the 1344 tumours initially described by Lauren, 53% were intestinal type, 33% were diffuse type, and the rest were indeterminate/ unclassified type.[57]

Intestinal carcinoma:

They have a glandular pattern usually accompanied by tubules, papillary formation or solid components. The glands range from well differentiated to moderately differentiated grade, sometimes with poorly differentiated picture. The glandular epithelium consists of pleomorphic cells with large hyperchromatic nuclei often with numerous mitoses. The adjacent gastric mucosa often shows chronic gastritis, intestinal metaplasia and sometimes dysplasia. It is common in the elderly and in males.

Diffuse carcinoma:

These are predominantly composed of poorly cohesive and diffusely infiltrating small tumour cells having indistinct cytoplasm and hyperchromatic nuclei. Glandular pattern may be seen in the more superficial part of the tumour. Signet ring cells are common along with extracellular mucin in the stroma. Desmoplasia is more pronounced and generally there is no accompanying dysplasia or metaplasia. They commonly occur at a younger age and shows equal sex incidence.

Classification of Mulligan and Rember (1975)[(58)]:

This classification expands Lauren's classification by adding a third type - pylorocardiac gland carcinoma[(58)]. Pylorocardiac gland carcinomas commonly present as well demarcated tumours. They are commoner in men and microscopically show varying sized glands with tubular or papillary pattern containing cells that often show striking vacuolation or clear cell change that stain brilliantly with periodic acid–Schiff reaction.

Ming's Classification (1977)[(59)]:

This classification divides gastric adenocarcinomas into two types - Expanding type and Infiltrating type[(59)]. The expanding type has pushing edges and forms discrete tumour nodules. This compares roughly to the

intestinal type of Lauren and occurs in patients over 50 years of age. The infiltrative type is ill defined, contains widely infiltrative tumour cells with poor inflammatory cell response and collagenous stroma. It is more common under the age of 50.

WHO Classification (1990) [(60)]***:***

WHO classification of Gastric tumours is given in Annexure II. Based on the predominant histological picture, gastric adenocarcinoma is classified as the following types.

Tubular adenocarcinoma :

It is composed predominantly of neoplastic tubules, showing irregular branching and anastomosis, surrounded by fibrous stroma. Individual tumour cells are cuboidal, columnar or flattened by intraluminal mucin. Cytological atypia varies from low to high-grade. A poorly differentiated variant called solid carcinoma is also seen. An oncocytic variant of tubular adenocarcinoma has also been described[(61)].

Papillary adenocarcinoma:

These show well-differentiated picture with elongated finger-like processes lined by cuboidal cells and central fibro-vascular connective tissue cores. Some cases show tubular differentiation (papillotubular). Rarely, micropapillary architecture can be seen. Typically these grow as a polypoid mass projecting into the lumen of the stomach.

Mucinous carcinoma:

Carcinomas containing large amounts of extracellular mucin in more than 50% of the tumour area are called as Mucinous carcinomas. In some cases, the cells form glands lined by columnar mucus-secreting cells (well differentiated type). In few others, disaggregated ribbons or clusters of cells floating in lakes of mucin are seen (poorly differentiated type). There may also be mucin in the inter-glandular stroma. Scattered signet-ring cells, if seen, do not dominate the histological picture. They commonly occur as polypoid, fungating or ulcerative growths.

Signet ring cell carcinoma:

These are carcinomas composed predominantly of single cells or small clusters of cells with intra-cytoplasmic mucus vacuoles which accounts for more than 50% of the tumour. These cells have nuclei which push against cell membranes forming a classical signet ring cell appearance. The cytoplasm contains acid mucin and stain with Alcian blue at pH 2.5. They also have cells with no mucin and cells with eosinophilic granular cytoplasm having neutral mucin. This tumour which is more common in younger patients usually occurs in the distal stomach. They usually infiltrate the wall of the stomach diffusely and are

accompanied by marked fibrosis giving rise to the linitis plastica appearance on macroscopic examination.

The Goseki Classification (1992) [(62)]:

According to the degree of tubular differentiation and the amount of intracellular mucin present, Goseki et al[(62)] proposed the following classification

Group I - has well differentiated tubules & poor intracellular mucin

Group II - has well differentiated tubules & plentiful intracellular mucin

Group III - has poorly differentiated tubules & poor intracellular mucin

Group IV - has poorly differentiated tubules & plentiful intracellular mucin

Carneiro Classification (1997) [(63)]:

Carneiro et al gave a much simpler system classifying the tumours into glandular, isolated cell carcinomas, solid variety and a mixed type showing mixture of glandular and isolated cell types.

The rare variants of gastric carcinoma include Squamous cell carcinoma [(64)], Adenosquamous Carcinoma[(65)],Small cell Carcinoma[(66)], Parietal cell carcinoma[(67)], Medullary carcinoma with lymphoid stroma[(68)], Hepatoid adenocarcinoma[(69)], Choriocarcinoma[(70)], Gastric carcinoma with rhabdoid features [(71)] and Carcinosarcoma.[(72)]

Grading Of Gastric Carcinoma:

Three tier grading system is used for grading gastric adenocarcinoma.

Well differentiated: shows well-formed glands, often resembling metaplastic intestinal epithelium.

Moderately differentiated: intermediate between well differentiated and poorly differentiated.

Poorly differentiated: shows highly irregular glands that are recognized with difficulty, or shows single cells that remain isolated or arranged in clusters with mucin secretions.

SPREAD OF GASTRIC CARCINOMA:

Direct spread after penetration through serosa to pancreas, liver, spleen, transverse colon and omentum occurs. Direct spread to lower part of esophagus and proximal part of duodenum is also seen. Lymphatic spread along the lesser & greater curvature lymphatics to left gastric, hepatic and celiac nodes occur. More distant spread to paraaortic and mesenteric nodes, through the thoracic ducts to supraclavicular nodes may also occur. Incidence of lymph node metastasis seem to increase with depth of invasion into the stomach wall.[(73)]

Haematogenous spread occurs mainly to liver, rarely to lung, adrenals, peritoneum and ovaries. Unusual sites such as kidney, spleen,

uterus and meninges are more often involved in diffuse type[74]. Transperitoneal spread is seen as secondary deposits in omentum, peritoneum and mesentery.

STAGING:

The TNM staging system[75] is widely used as it is the best available predictor of prognosis and the recent TNM staging proposed by AJCC is given in Annexure III.

PROGNOSIS:

The prognosis of gastric carcinoma varies from country to country. The overall survival rate in the Western countries is 4 to 13%[76] which is poor compared to Japan which shows the best results with an overall 5-year survival rate of 89% for early carcinoma and 46% for advanced carcinoma. This is atleast partly by the greater frequency of superficial carcinomas, and aggressive Japanese surgical approach to treatment with extensive and meticulous lymph node dissection[77]. Different criteria in the differential diagnosis of severe dysplasia and carcinoma between Japan and Western countries may also contribute for some of the differences[78].

PROGNOSTIC FACTORS:

Any variable that provides useful information in assessing the outcome of a disease at the time of diagnosis are called as prognostic

factors. They may be clinical factors, morphological factors and/or genetic / molecular factors.

The clinical factors which indicate poor prognosis are young age and proximal location of gastric cancers[76]. The 5- year survival rates of the cardia tumours are under 20%[79] and the median survival is only about 7 months[80].

The pathological factors play a more useful role in assessing prognosis. A few important ones are the following:

1. *Tumour size:* Small size is associated with a better prognosis but this is closely linked to depth of penetration[81]

2. Tumour stage: This is the most significant prognostic factor. Depth of invasion is considered in staging which is directly proportional to the chance of distant metastasis[82].

3. *Microscopic type and grading:* Intestinal type tumours in Lauren's classification has relatively better prognosis than diffuse types[83].

4. *Lymphocytic response:* Presence of inflammatory infiltrate at tumour and normal tissue interface is associated with good patient survival[84].

5. Lymphovascular invasion: Indicates infiltration of tumour cells into vascular spaces. It predicts the risk of recurrence and distant metastasis.

6. Perineural invasion: When present has poor prognosis when compared to negative cases[85].

7. Regional lymph node involvement: When nodal involvement is present, 5-year survival rate drops to below 10% and it is 50% in the node negative cases. The number of nodes involved is also significant. Overall survival rate decreases as the number of positive node increases[86].

Other factors found to have poor prognosis are tumour necrosis, infiltrative margins of tumour and involvement of surgical margins.

Many molecular biomarkers are identified which play an important prognostic role in gastric carcinoma management. DNA aneuploidy has been reported in about 40–50% of gastric carcinomas. It has been found that aneuploid tumours are significantly associated with both lymph node and distant metastases and show lower survival rates compared to diploid cancers[87]. Over expression of HER-2/Neu which is a transmembrane epidermal growth factor receptor protein is reported to have poorer outcome[88], but some studies showed no such association[127]. Studies based on immunohistochemistry showed that p53 protein over expression is associated with decreased survival[90] but some studies failed to confirm this[91]. E-cadherin, a transmembrane protein plays a significant role in maintenance of intercellular connections. Germline mutations in E-cadherin gene are associated with cancers of diffuse type and aggressive behaviour[92]. Other factors like increased expression of p27kip1[93],

cathepsin D[94], loss of Fhit protein[95] and increased proliferation indices[96] are shown to be associated with reduced survival.

E Cadherin:

The malignant potential of cancer manifests by the ability of tumour cells to spread from primary site and form metastases in distant regions. A number of different steps in the complex metastatic process show alterations in the adhesive properties of tumour cells [97-99]. One of the major molecules mediating adhesion between epithelial cells is the calcium-dependent cell adhesion molecule E Cadherin. This 120-kilodalton transmembrane glycoprotein, is predominantly localized to the lateral cell border and is associated with the contractile cytoskeleton, mediates homotypic adhesion & plays an important role in organization and maintenance of the tissue structure [100-102]. The selective loss of E-cadherin expression or function is shown to be associated with changes in cellular phenotype, with the development of invasive behaviour of tumour cells, effects which can be reversed by transfection of E-cadherin-encoding cDNA[103,104].

Results of immunohistochemical investigations indicate that E Cadherin expression is decreased in various human carcinomas in vivo[105-108]. The frequency of reduced expression of E Cadherin in various studies range from 32 to 92% and conflicting results were found. For

example, Shiozaki et al[109] reported a reduced E-cadherin expression in undifferentiated tumours, Shimoyama and Hirohashi[110] could find no correlation between E-cadherin expression and the differentiation of the tumour and, in fact, observed that nearly two-thirds of the undifferentiated gastric carcinomas investigated were E-cadherin positive. Level of E Cadherin expression can be measured by measuring the level of soluble E Cadherin in serum of the patient or by immunohistochemical staining of the tissues. The scoring system used for measuring E Cadherin expression using immunohistochemistry is given in Annexure V.

EGFR:

The epidermal growth factor receptor (EGFR) gene, also called Erb B, is located at chromosomal region 7p12. It encodes a 170-kDa transmembrane tyrosine kinase receptor, which is a member of the EGFR family[112]. EGFR is activated by binding to its ligands (epidermal growth factor or transforming growth factor-alpha), which results in homodimerization or heterodimerization with another member of EGFR family[113]. This causes receptor activation and is followed by phosphorylation of specific tyrosine residues within the cytoplasmic tail, which in turn stimulates the downstream signalling pathway which regulates cell proliferation, migration, adhesion, differentiation and

survival[113]. The frequency of EGFR over expression and / or amplification in Gastric carcinoma has been variously reported as ranging from 18 to 44%.[114-116]. Recently, the new therapeutic agents targeting EGFR has attracted attention. They either bind to extracellular domain of the receptor (eg:cetuximab, matuzumab and panitumumab), where they compete with the natural ligand binding to the receptor and block activation of that receptor or they compete with adenosine triphosphate binding to the tyrosine kinase portion of endodomain of the receptor and they abrogate the receptor's catalytic activity (eg: gefitinib, erlotinib and lapatinib). Both groups of compounds are effective in blocking the downstream receptor-dependant signaling pathway. For patients with gastric cancer, a few clinical trials have been performed with these drugs but with ambiguous results. One clinical trial has found that a subpopulation of Gastric carcinoma patients showed evidence of gefitinib sensitivity. Accurate evaluation of the EGFR protein and/or EGFR gene expression status in patients with Gastric carcinoma is important for determining patient's eligibility for new targeted therapy.

Increased expression of this EGFR protein can be studied by either Immunohistochemical method or by Fluorescent In Situ Hybridization (FISH) method. Immunohistochemically a positive reaction is considered

in the presence of brown transmembrane immunostaining and the scoring system used to evaluate expression of EGFR is given in Annexure V.

p53:

The human TP53 gene was cloned in 1985 and its character as a tumour suppressor gene was discovered in 1989 by Bert Vogelstein. It is considered the "Guardian of the genome" and is located on the 17p chromosome, coding for a protein of 53 kD. The role of p53 is central in cell - cycle regulation, DNA repair and cell apoptosis. The production of p53 is increased in response to cellular insults or DNA damage which then induces cell - cycle arrest at the G1/S junction. Thus, p53 is essential for control of tumour growth, apoptosis & maintaining genome stability. Normal p53 protein is rapidly removed from the nucleus, but mutant form has a prolonged half-life, leading on to intranuclear accumulation, thus detectable immunohistochemically. Correlation between the over expression of p53 and the poor prognosis and survival of patients have been observed in several studies but several other studies have failed to confirm this[120.121]. Overall prevalence of p53 immunoreactivity in advanced gastric carcinoma ranges from 35 to 65% as shown by several studies. Over expression of p53 protein is more common in intestinal-type carcinomas than in diffuse tumours[119]. Most commonly used methods for

detection of these mutations are immunohistochemistry, flow-cytometry, PCR and genomic sequencing. Immunohistochemically positive reaction is considered when brown nuclear immunostaining is observed and the scoring system used to identify p53 positive cases by Immunohistochemistry is given in Annexure V.

HER-2/Neu:

HER-2/Neu oncogene, also known as c-erbB- 2, encodes a transmembrane tyrosine kinase receptor, homologous to epidermal growth factor receptor[123]. HER molecule belongs to a family of glycoproteins which consist of an extracellular domain for binding ligands, a lipophilic transmembrane domain, and an intracellular domain which carries tyrosine kinase activity. HER-2/Neu gene, located on chromosome 17q21, is related to the oncogene v-erbB of the avian erythroblastosis virus. Protein encoded by this gene – p185 – is a growth factor receptor involved in growth and progression of malignant cells. The prognostic value of c-erbB-2 has been mainly shown in breast cancer in which patients with over expression of this gene have a significantly lower relapse free and overall survival rate compared to patients without over expression [124,125]. There are also studies suggesting that over expression of this protein is a new, independent prognostic factor for

overall survival in gastric cancer[126]. But, some studies have failed to find an association with prognosis [127,128]. Also, with the availability of the monoclonal antibody trastuzumab, HER-2/Neu can be the target of therapy, adding to the importance of research on HER-2/Neu. Immunohistochemistry and fluorescence in situ hybridization (FISH) are the techniques routinely recommended for determining HER-2/Neu status.

Immunohistochemically, a positive reaction is considered in the presence of brown transmembrane immunostaining and the scoring system to identify HER-2/Neu over expression is given in Annexure V.

Immunohistochemistry:

Albert Coons et al in 1941 first labelled antibodies directly with fluorescent isocyanate. Nakane and Pierce et al in 1966, introduced indirect labelling technique in which unlabelled antibody is followed by second antibody or substrate. Various stages of development of Immunohistochemistry include peroxidase – antiperoxidase method (1970), alkaline phosphatase labelling (1971), avidin biotin method (1977) and two layer dextrin polymer technique (1993) [129].

Antigen Retrieval:

Antigen retrieval can be done by the following different techniques to unmask the antigenic determinants of fixed tissue sections.

1. Proteolytic enzyme digestion

2. Microwave antigen retrieval

3. Pressure cooker antigen retrieval

4. Microwave and trypsin antigen retrieval

Proteolytic Enzyme Digestion:

Huank et al in 1976 introduced this technique to breakdown formalin cross linkages and to unmask the antigen determinants. The most commonly used enzymes include trypsin and proteinase[130]. The disadvantages include over digestion, under digestion and antigen destruction.

Microwave Antigen Retrieval:

This is a new technique most commonly used in current practice. Microwave oven heating involves boiling formalin fixed paraffin sections in various buffers for rapid and uniform heating [129].

Pressure Cooker Antigen Retrieval:

Miller et al in 1995 compared and proved that pressure cooking method has fewer inconsistencies, less time consuming and can be used to retrieve large number of slides than in microwave method[131].

Pitfalls of Heat Pre-treatment:

Drying of sections at any stage after heat pre-treatment destroys antigenicity. Nuclear details are damaged in poorly fixed tissues. Fibers

and fatty tissues tend to detach from slides while heating. Not all antigens are retrieved by heat pre-treatment and also some antigens like PGP 9.5 show altered staining pattern.

***Detection Systems**:*

After addition of specific antibodies to the antigens, next step is to visualize the antigen antibody reaction complex. The methods employed are direct and indirect methods. In the direct method, primary antibody is directly conjugated with the label. Most commonly used labels are flourochrome, horse radish peroxidase and alkaline phosphatase. Indirect method is a two-step method in which labelled secondary antibody reacts with primary antibody bound to specific antigen. The use of peroxidase enzyme complex or avidin biotin complex further increases the sensitivity of immunohistochemical stains[(129)]. In 1993, Pluzek et al introduced enhanced polymer one step staining, in which large numbers of primary antibody and peroxidase enzymes are attached to dextran polymer back bone. This is the rapid and sensitive method[(132)]. Dextran polymer conjugate two step visualization system is based on dextran technology in Epos system. This method has greater sensitivity and is less time consuming.

MATERIALS AND METHODS

This study is a prospective study of gastric carcinoma conducted in the Institute of Pathology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai during the period of January 2011 to December 2011. A total of 10,357 cases were received in our Surgical Pathology department during January 2011 to December 2011. Of these, 677 were gastric specimens including 578 endoscopic biopsies and 99 gastrectomy specimens. Of the 677 gastric specimens, 321 were non neoplastic, 4 were benign and 352 were malignant cases. Of the 352 malignant cases (including 254 endoscopic biopsies and 98 gastrectomy specimens) 338 were adenocarcinoma, 8 were lymphoma, 5 were GIST, 1 was neuroendocrine tumour.

Source of Data

Endoscopic biopsy from stomach as well as resected specimens (subtotal, total, radical and palliative gastrectomy) from the Department of Surgery, Surgical Gastroenterology, Surgical Oncology and Geriatric surgery, Government General Hospital which were received in Institute of Pathology, Madras Medical College during the period of January to December 2011 and reported as adenocarcinoma were included for the study. A total 667 gastric specimens were received during January to

December 2011, out of which 338 cases were reported as adenocarcinoma.

Inclusion criteria

All gastric adenocarcinoma cases reported in both endoscopic biopsies as well as resected specimens, irrespective of age and sex were included for the study.

Exclusion criteria

- Non neoplastic lesions and benign tumours of stomach.
- Malignancies other than adenocarcinoma.
- Tumours located in gastroesophageal junction.
- Gastrectomies performed for reasons other than gastric tumours.

Method of data collection

Detailed history of the cases regarding age, sex, clinical presentation, investigations done along with the findings, type of procedure done were obtained for all the gastric specimens received during the period of study. Haematoxylin and Eosin stained 4 micron thick sections of the paraffin tissue blocks of the cases were prepared and cases reported as gastric adenocarcinoma were selected. Among the 338 adenocarcinoma cases reported, 50 patients (25 endoscopic biopsies and 25 resected specimens) attending the medical oncology OP for follow up were selected for Immunohistochemical analysis.

Variables studied

The following clinical and pathological parameters were evaluated. Age, gender, size, location (Eso-cardia, body, pyroloantrum, pangastric), gross appearance (ulcerative, nodular, ulceroproliferative, diffuse), histological types (diffuse, signet ring cell, tubular, papillary, mucinous), Lauren's classification (intestinal, diffuse, indeterminate), depth of infiltration (T1 -invasion of mucosa and submucosa,T2- invasion of muscularis mucosa,T3- invasion of subserosa, T4-invasion of serosa or adjacent organs), grade (well differentiated, moderately differentiated, poorly differentiated). The resected specimens were evaluated for presence of lymphatic invasion, vascular invasion, perineural invasion, and lymphocytic response. 25 endoscopic biopsies (unresectable cases with locally advanced stage or distant spread) and 25 resected specimens of patients attending the medical oncology department for follow up and further treatment were selected and their representative formalin fixed paraffin embedded tissue samples were subjected to immunohistochemical analysis with a panel of 4 markers i.e., E Cadherin, EGFR, p53 and HER-2/Neu.

Immunohistochemical evaluation

Antigen	Vendor	Species(clone)	Dilution	Positive control
E Cadherin	Biogenex	Mouse	Ready to use	Stomach
EGFR	Biogenex	Mouse	Ready to use	Stomach
p53	Biogenex	Mouse	Ready to use	Stomach
HER-2/Neu	Biogenex	Mouse	Ready to use	Stomach

Immunohistochemical analysis was done in paraffin embedded tissue samples using supersensitive polymer HRP system based on non-biotin polymeric technology. 4 micron thick sections from formalin fixed paraffin embedded tissue samples were transferred onto gelatin coated slides. Heat induced antigen retrieval was done. The antigen was bound with mouse monoclonal antibody (Biogenex) against E Cadherin, EGFR, p53 and HER-2/Neu proteins and then detected by the addition of secondary antibody conjugated with horse radish peroxidase polymer and diaminobenzidine substrate. Step by step procedure of Immunohistochemistry is given in Annexure IV.

Interpretation and scoring

The immunohistochemically stained slides were analyzed for the presence of reaction, cellular localization, percentage of cells stained and intensity of staining. Cytoplasmic membrane staining was assessed for E Cadherin, EGFR, and HER-2/Neu and nuclear staining was assessed for

p53. Details of the scoring pattern applied to classify positive/ negative cases for HER-2/Neu, p53, EGFR and reduced expression/ normal expression in case of E Cadherin is given in Annexure V.

Patients follow up

Mean follow-up time was 11.5 months, with a range of 2 months to 20 months. To eliminate bias due to deaths directly resulting from complications of operation patients who died within 6 weeks of surgery were excluded from the statistical analysis of survival.

Data entry

All the data collected and the results obtained were entered into Excel 2007.

Statistical analysis

The statistical analysis was performed using statistical package STATA 11(Statacorp). For continuous variables like age and size of tumour, Student T test was done to obtain the results. For discrete variables like gender, gross appearance, histological type, Lauren's classification, grade, depth of infiltration, lymphovascular invasion, perineural invasion and survival, Pearson chi square test and Fisher's exact test were used to obtain results. Reports were expressed in percentage or odds ratio with 95% as CI. P value of 0.05 was taken as cut off point to determine statistically significant results.

OBSERVATION AND RESULTS

During January to December 2011 a total of 10,357 cases were received for histopathological examination in the Institute of Pathology, Madras Medical College. Among the 667 gastric specimens, 352 were malignant cases. Among the malignant cases, 338 were adenocarcinoma, 8 were lymphoma, 5 were GIST and 1 was neuroendocrine tumour. Adenocarcinoma of stomach constituted for 52.77% of all the gastric specimens received and 3.26% of all total cases received during 2011.

Gastric carcinoma had a peak incidence in the age group of 55 to 64 years. The oldest age of presentation was 84 years and youngest age of presentation was 21 years. Among the 338 cases 244 (72.19%) were males and 94 cases (27.81%) were females. Gastric cancer in this study showed increased incidence among 55 to 64 years and overall male preponderance (Table 1 and Chart1)

Table 1: Age and Sex Distribution of study participants

Age	**Males (%)**	**Females (%)**	**Total (%)**
0 to 40 years	24 (7.1)	25 (7.4)	49 (14.50)
41 to 54 years	71 (21)	31 (9.2)	102 (30.18)
55 to 64 years	92 (27)	20 (6)	112 (33.14)
65 and above	57 (16.9)	18 (5.4)	75 (22.18)
Total	244 (72)	94 (28)	338 (100)

The mean age of incidence of gastric carcinoma was higher in males (56.49 years) than that of females (50.87 years) and this difference in age incidence was found to be statistically significant. (Table2)

Table2: Mean age of Incidence in males and females

Sex	No of Cases	Mean of age(yrs)	Std deviation	Mann Whitney test
Male	244	56.49	.708	**P Value**
Female	94	50.87	1.415	**0.003**

Of the 338 cases, increased occurrence (61.83%) was noted in the pyloroantral region. (Figure 3 & Chart 2)

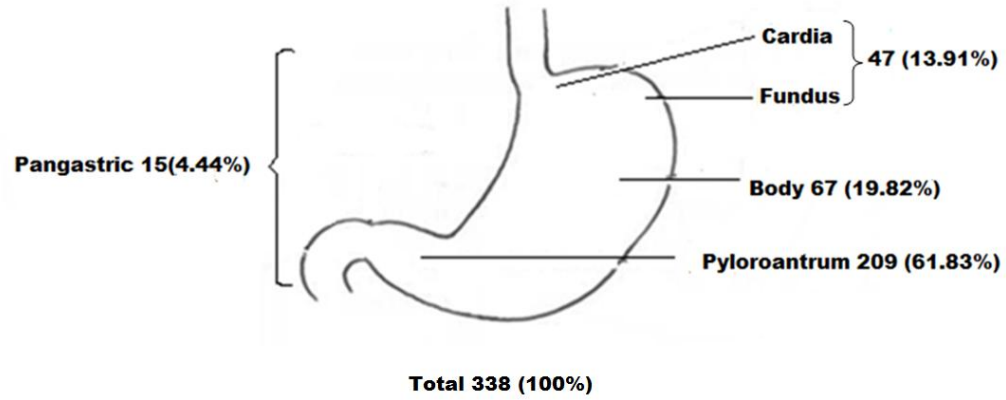


Figure 3: Site wise Distribution of gastric cancer

Ulceroproliferative type (58.88%) was the most common gross appearance, followed by ulcerative type (26.63%), nodular type (10.36%) and diffuse type (4.13%). (Figure 4 & Chart 3)

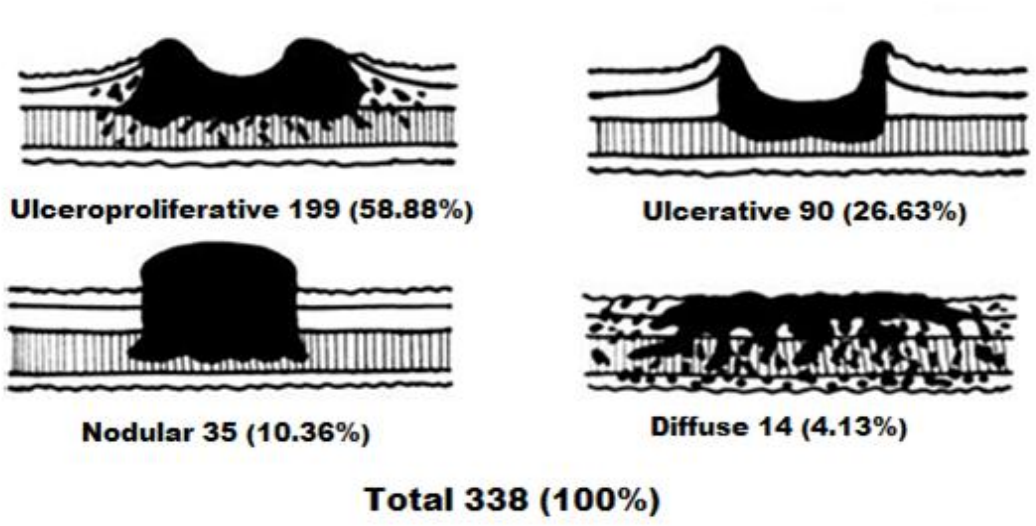


Figure 4: Distribution of various gross types of gastric cancer

Among the various histological subtypes, tubular type of gastric carcinoma was the most common. (Table 3 & Chart 4)

Table 3: Distribution of Gastric Cancer based on Histological Subtype

Histological subtype	No of cases (%)
Tubular	189 (55.91)
Diffuse	71 (21.01)
Signet ring cell	44 (13.02)
Mucinous	19 (5.62)
Papillary	15 (4.44)
Total	338 (100)

On applying Lauren's classification, of the 338 cases, 205 were intestinal type, 127 were diffuse type and 8 were indeterminate. (Table 4 & Chart 5)

Table 4: Distribution of Gastric Cancer based on Lauren's Classification

Lauren's type	No of cases (%)
Intestinal	205 (60.6)
Diffuse	127 (37.5)
Indeterminate	6 (1.9)
Total	338 (100)

Depending on the histological grading, distribution of the gastric cancers were as shown in Table 5 & Chart 6

Table 5: Distribution of Gastric Cancer based on Histological Grading

Histological grade	**No of cases (%)**
Well differentiated	62 (18.34)
Moderately differentiated	113 (33.43)
Poorly differentiated	163 (48.23)
Total	338 (100)

Depth of infiltration was studied in the 93 resected specimens (total and subtotal gastrectomies). 3 cases showed invasion up to submucosa (T1), 17 cases showed invasion into muscular layer (T2), 55 cases showed invasion into subserosa (T3), 19 cases showed invasion into serosa or adjacent structures (T4).(Figure 5 & Chart 7)

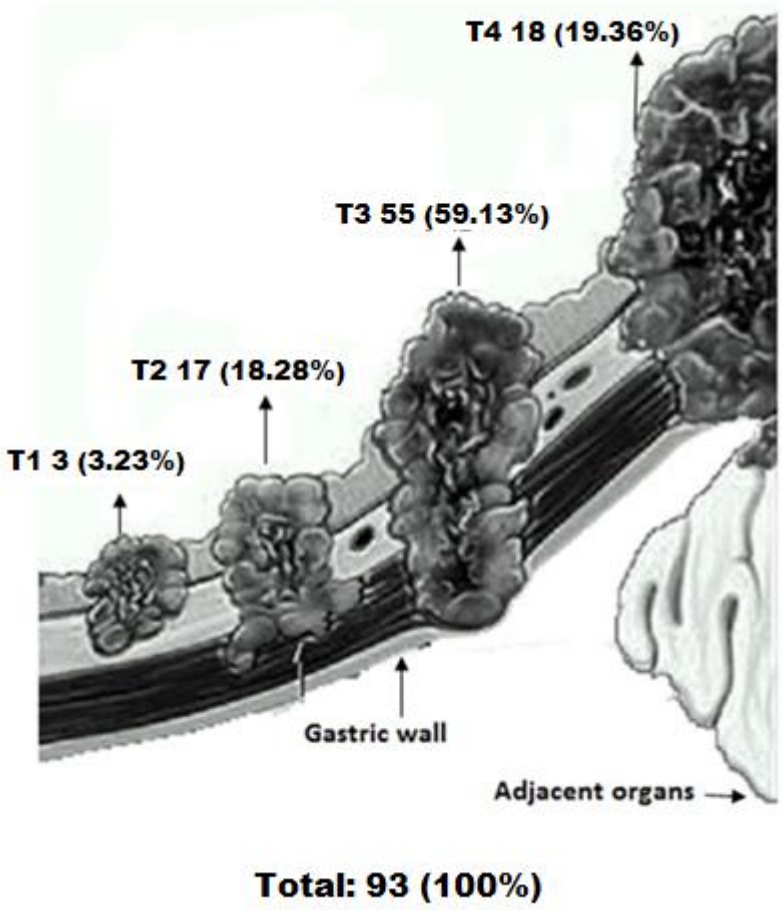


Figure 5: Distribution according to Depth of Infiltration

Size of the tumour was studied in the 93 resected specimens, and the distribution was as shown in Figure 6 & Chart 8.

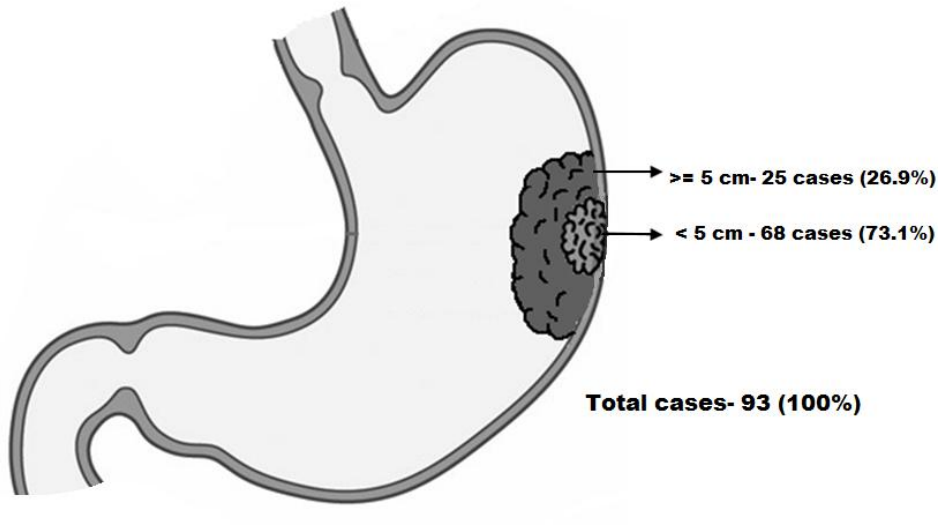


Figure 6: Distribution of Gastric Cancer according to size

Of the 93 resected specimens 80.65% cases showed lymphatic infiltration, 59.54% cases showed vascular invasion. Perineural invasion was seen only in 34.41% of cases. Lymphocytic response was seen in 39.48% cases. (Table 6 & Chart 9)

Table 6: Distribution of Other Prognostic Factors in Gastric Carcinoma

Prognostic factor	**Present (%)**	**Absent (%)**	**Total (%)**
Lymphatic invasion	75 (80.65)	18 (19.35)	93 (100)
Vascular invasion	55 (59.14)	38 (40.86)	93 (100)
Perineural invasion	32 (34.41)	61 (65.59)	93 (100)
Lymphocytic response	37 (39.78)	56 (60.22)	93 (100)

Results of Immunohistochemical analysis:

Of the 338 cases, a total of 50 cases comprising 25 resected specimens and 25 endoscopic biopsies were selected and subjected to a panel of 4 immunohistochemical markers, i.e., E Cadherin, EGFR, p53 and HER-2/Neu.

Among the 50 cases, 29 (58%) cases showed reduced expression for E Cadherin, 13 (26%) cases showed EGFR over expression, 27 (54%) cases showed positivity for p53, and 10 (20%) cases showed HER-2/Neu over expression. (Table 7 & Chart 10)

Table 7: Percentage of Expressions of E Cadherin, EGFR, p53 and Her2/ Neu in Gastric Cancer:

IHC Para-Meter	**E Cadherin (%)**		**EGFR (%)**		**p53 (%)**		**HER-2/Neu (%)**	
Result (%)	*Reduced expression*	*Normal expression*	*Positive*	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*
	29 (58)	21 (42)	13(26)	37 (74)	27(54)	23 (46)	10(20)	40 (80)
Total	50 (100)		50 (100)		50 (100)		50 (100)	

Correlation of E Cadherin with Various Clinicopathological Factors:

The mean age of patients with normal E Cadherin expression was 54.57 years and that of patients with reduced E Cadherin expression was 53.89 years. There was no significant difference in the age at presentation between the two groups (Table 8 & Chart 11)

Table 8: Association of age of patient with E Cadherin expression

E Cadherin	**No of cases**	**Mean age in years**	**Std deviation**	**Mann Whitney test**
Reduced expression	29	53.89	10.65	P Value 0.837
Normal expression	21	54.57	12.47	

Of the cases showing reduced E Cadherin expression, 17 were males and 12 were females and there was no significant difference in sex wise distribution of gastric cancer showing reduced E Cadherin expression. (Table 9 & Chart 12)

Table 9: Association of Gender with E Cadherin Expression

Sex	**Male (%)**	**Female (%)**	**Total (%)**
Reduced expression	17 (58.62)	12 (41.38)	29 (100)
Normal expression	12 (57.14)	9 (42.86)	21 (100)
Total No. of Cases	29 (58)	21 (42)	50 (100)
Pearson chi square test	P value 0.917		

In the present study, among the 29 cases showing reduced E Cadherin expression, increased frequency (65.54%) of cases were from pyloroantral region but the association was not statistically significant. (Table 10 & Chart 13)

Table 10: Association of Site with E Cadherin Expression

Site	**Eso-Cardia (%)**	**Body (%)**	**Pyloro antrum(%)**	**Pan-gastric(%)**	**Total (%)**	**Fisher's Exact test**
Reduced expression	6 (20.68)	1(3.44)	19 (65.54)	3 (10.34)	29(100)	P Value
Normal expression	3(14.28)	0	16 (76.2)	2 (9.52)	21(100)	0.751
Total No. of Cases	9(18)	1(2)	35 (70)	5 (10)	50(100)	

On correlating with gross appearance, among the 29 cases showing reduced E Cadherin expression, 19 cases (65.5%) were ulceroproliferative type but this association was not statistically significant. (Table11 & Chart 14)

Table 11: Association of Gross Appearance of Tumour with E Cadherin Expression

Gross type	Ulcerative (%)	Nodular (%)	Ulcero-Proliferative (%)	Diffuse (%)	Total (%)	Fisher's exact test
Reduced expression	5 (17.24)	2 (6.89)	19 (65.51)	3 (10.36)	29(100)	
Normal expression	3 (14.28)	3 (14.28)	13 (61.90)	2 (9.54)	21(100)	P value 0.858
Total No. of Cases	8 (16)	5 (10)	32 (64)	5 (10)	50(100)	

Among the 25 resected specimens for which expression of E Cadherin was studied, 6 cases were showing reduced E Cadherin expression, among which 4 cases (66.66%) were less than 5 cm. There was no significant association between size of the tumour and E Cadherin expression. (Table 12 & Chart 15)

Table 12: Correlation of Tumour Size with E Cadherin Expression

Size	<5cm (%)	>=5cm (%)	Total (%)	Fisher's exact test
Reduced expression	4(66.67)	2(33.33)	6(100)	
Normal expression	15(78.95)	4(21.05)	19(100)	P Value 0.428
Total No. of Cases	19(76)	6(24)	25(100)	

On correlating with the histological types, higher number (55.2%) of cases with reduced E Cadherin expression was seen in tubular type. There was no significant association between histological type and E Cadherin expression. (Table 13 & Chart 16)

Table 13: Association of Histological Type with E Cadherin Expression

Histological type	**Diffuse type (%)**	**Signet ring cell type (%)**	**Tubular type (%)**	**Papillary type (%)**	**Mucinous type (%)**	**Total (%)**	**Fisher s exact test**
Reduced expression	7 (24.2)	4 (13.8)	16(55.2)	0	2 (6.8)	29 (58)	P Value 0.314
Normal expression	2 (9.5)	1 (4.8)	16(76.1)	1 (4.8)	1 (4.8)	21 (42)	
Total No. of Cases	9 (18)	5 (10)	32 (64)	1 (2)	3 (6)	50(100)	

On comparing and analysis using Lauren's classification, greater frequency of reduced expression was noted with intestinal type (55.2%) than diffuse type (44.8%) but the association was not statistically significant. (Table14 & Chart 17)

Table 14: Association of Lauren's Classification with E Cadherin Expression

Lauren's	Intestinal type (%)	Diffuse type (%)	Total (%)	Fisher's exact test
Normal expression	17 (80.9)	4 (19.1)	21 (100)	P value 0.058
Reduced expression	16 (55.2)	13 (44.8)	29 (100)	
Total No. of Cases	33 (66)	17 (34)	50 (100)	

Reduced expression of E Cadherin was seen to be more with increasing tumour grade. Among the cases showing reduced E Cadherin expression, 13.8% were well differentiated, 27.5% were moderately differentiated and 58.7% were poorly differentiated grades. (Table 15 & Chart 18)

Table 15: Association of Tumour Grade with E Cadherin Expression

Grade	Well Differentiated (%)	Moderately Differentiated (%)	Poorly Differentiated (%)	Total (%)	Fisher's exact test
Reduced expression	4 (13.8)	8 (27.5)	17 (58.7)	29(100)	P Value 0.208
Normal expression	5 (23.8)	9 (42.8)	7 (33.4)	21(100)	
Total No. of Cases	9 (18)	17 (34)	24 (48)	50(100)	

On correlating with the depth of infiltration for the 25 resected specimens for which E Cadherin expression was studied, among the cases showing reduced E Cadherin expression, increased frequency (66.67%) of cases showed infiltration up to T3 level but the association was not statistically significant. (Table16 & Chart 19)

Table 16: Association of Depth of Infiltration with E Cadherin Expression

Depth	**T2 (%)**	**T3 (%)**	**T4 (%)**	**Total (%)**	**Fisher's exact test**
Reduced Expression	1 (11.11)	6 (66.67)	2 (22.22)	9 (100)	P value 0.489
Normal Expression	2 (12.50)	13 (81.25)	1 (6.25)	16 (100)	
Total No. of Cases	3 (12)	19 (76)	3 (12)	25 (100)	

Among the 25 resected specimens for which E Cadherin expression was studied, 9 cases showed reduced expression. Of these 9 cases, 92.9% of cases had lymphatic invasion, 77.8% cases had vascular invasion, 66.7% cases had perineural invasion and only 11.1% cases had lymphocytic response but these associations were not statistically significant. (Table 17 & Charts 20 to 23).

Table 17: Association of other Prognostic Markers with E Cadherin Expression

Patient Character-istic	Lymphatic Invasion (%)		Vascular Invasion (%)		Perineural Invasion (%)		Lymphocytic Response (%)	
	Absent	*Present*	*Absent*	*Present*	*Absent*	*Present*	*Absent*	*Present*
Reduced Expression	1 (7.1)	13(92.9)	2(22.2)	7 (77.8)	3(33.3)	6 (66.7)	8 (88.9)	1 (11.1)
Normal Expression	3(27.3)	8 (72.7)	6(37.5)	10(62.5)	8 (50)	8 (50)	8 (50)	8 (50)
Fisher's exact test	P Value 0.073		P Value 0.366		P Value 0.444		P Value 0.141	

Among the 50 cases followed up for a mean period of 11.5 months, 79.3% with reduced expression were dead and only 20.7% with reduced expression were alive and the association was found to be statistically significant. (Table 18 & Chart 24)

Table 18: Association of Survival of patient with E Cadherin Expression

Survival of patient	Alive (%)	Dead (%)	Total (%)	Pearson chi square test
Reduced expression	6 (20.7)	23 (79.3)	29 (100)	**P Value 0.004**
Normal expression	12 (57.1)	9 (42.9)	21 (100)	
Total No. of Cases	18 (36)	32 (64)	50 (100)	

Correlation of EGFR with Various Clinicopathological Factors:

The mean age of patients showing EGFR over expression was 55.76 years and that of patients without EGFR over expression was 53.62 years. No significant association was found between age of presentation and EGFR over expression. (Table 19 & Chart 11)

Table 19: Association of age of patient with EGFR Expression

EGFR	**No of cases**	**Mean of age in years**	**Std deviation**	**Mann Whitney test**
Negative	37	53.62	11.6	P Value 0.562
Positive	13	55.76	10.9	

Among the cases showing EGFR over expression, 64.3% were males and 35.7% were females and there was no significant difference in sex wise distribution of gastric cancer showing EGFR over expression. (Table 20 & Chart 12)

Table 20: Correlation of Gender with EGFR Expression

Sex	**Male (%)**	**Female (%)**	**Total (%)**
Negative	20 (54.1)	17 (45.9)	37 (100)
Positive	9 (64.3)	4 (35.7)	13 (100)
Total No of Cases	29 (58)	21 (42)	50 (100)
Fisher's Exact test	P Value 0.340		

Among the cases showing EGFR over expression, increased frequencies of cases (92.4%) were from pyloroantral region and the distribution was not statistically significant. (Table 21 & Chart 13)

Table 21: Association of Site of tumour with EGFR Expression

Site	**Eso Cardia (%)**	**Body (%)**	**Pyloroantrum (%)**	**Pangastric (%)**	**Total (%)**	**Pearson chi square test**
Negative	8(21.6)	1(2.7)	23(62.7)	5(13)	37 (100)	P Value
Positive	1(7.6)	0	12(92.4)	0	13 (100)	0.285
Total No of Cases	9(18)	1(2)	35(70)	5(10)	50(100)	

Among the cases with EGFR over expression, 69.2% were ulceroproliferative type, 15.4% were nodular type, 15.4% were ulcerative type, and none of the cases were of diffuse type. Association of EGFR over expression with gross appearance was not found to be statistically significant. (Table 22 & Chart 14)

Table 22: Association of Gross Appearance of Tumour with EGFR Expression

Gross Type	Ulcerative (%)	Nodular (%)	Ulcero Prolifer -ative(%)	Diffuse (%)	Total (%)	Fisher's exact test
Negative	6 (16.2)	3 (8.1)	23 (62.2)	5 (13.5)	37 (100)	P Value 0.858
Positive	2 (15.4)	2 (15.4)	9 (69.2)	0	13 (100)	
Total No of Cases	8 (16)	5 (10)	32 (64)	5 (10)	50(100)	

Of the 25 resected specimens for which EGFR expression was studied, 8 cases showed over expression, among which 75 % were less than 5 cm in size but the association was not statistically significant. (Table 23 & Chart 15)

Table 23: Association of Tumour Size with EGFR Expression

Size	<5cm (%)	>=5cm (%)	Total (%)	Fisher's exact test
Negative	13 (76.5)	4 (23.5)	17 (100)	P Value 0.668
Positive	6 (75)	2 (25)	8 (100)	
Total No of Cases	19 (76)	6 (24)	25 (100)	

On correlating with histological type, among the cases showing EGFR over expression, higher number of cases (76.2%) showed tubular type and the distribution was not statistically significant. (Table 24 & Chart 16)

Table 24: Association of Histological Type with EGFR Expression

Histologi-cal type	Diffuse type (%)	Signet ring cell type (%)	Tubular type (%)	Papillary type (%)	Mucinous type (%)	Total (%)	Fisher's exact test
Negative	7 (24.1)	4 (13.8)	16 (55.2)	0	2 (6.9)	29(100)	P Value 0.154
Positive	2 (9.4)	1 (4.8)	16 (76.2)	1 (4.8)	1 (4.8)	21(100)	
Total No of Cases	9 (18)	5 (10)	32 (64)	1 (2)	3 (6)	50(100)	

Among the 13 cases showing EGFR over expression, 76.9% were intestinal type and only 23.1% were of diffuse type and the association was not statistically significant. (Table25 & Chart 17)

Table 25: Association of Lauren's Classification with EGFR Expression

Lauren's	Intestinal type (%)	Diffuse type (%)	Total (%)	Fisher's exact test
Negative	23 (62.7)	14 (37.3)	37 (100)	P value 0.334
Positive	10 (76.9)	3 (23.1)	13 (100)	
Total No of Cases	33 (66)	17 (34)	50 (100)	

Among the cases showing EGFR over expression, higher number of cases showed moderately differentiated grade (61.5%) and the association was found to be statistically significant (Table 26 & Chart 18)

Table 26: Association of Tumour Grade with EGFR Expression

Grade	**Well Differentiated (%)**	**Moderately Differentiated (%)**	**Poorly Differentiated (%)**	**Total (%)**	**Fisher's exact test**
Negative	7 (18.9)	9 (24.4)	21 (56.7)	37(100)	**P value 0.044**
Positive	2 (15.4)	8 (61.5)	3 (23.1)	13(100)	
Total No of Cases	9 (18)	17 (34)	24 (48)	50(100)	

On correlating with the depth of infiltration for the 25 resected specimens for which EGFR expression was studied, 66.66% of cases with EGFR over expression were showing infiltration up to T3 level, 22.23% were showing infiltration up to T4 level and only 11.1% were showing infiltration up to T1 level. No statistically significant association between depth of infiltration and EGFR expression was found. (Table 27 & Chart 19).

Table 27: Association of Depth of Infiltration with EGFR Expression

Depth	**T2 (%)**	**T3 (%)**	**T4 (%)**	**Total (%)**	**Fisher's exact test**
Negative	2 (12.5)	13 (81.3)	1 (6.2)	16 (100)	P value 0.498
Positive	1 (11.11)	6 (66.67)	2 (22.22)	9 (100)	
Total No of Cases	3 (12)	19 (76)	3 (12)	25 (100)	

77.8% of cases with EGFR over expression showed lymphatic invasion, 44.4% of case with EGFR over expression showed vascular invasion, 55.6% of cases with EGFR over expression showed perineural invasion and 44.4% of cases with EGFR over expression showed lymphocytic response. None of these associations were statistically significant. (Table 28 & Charts 20 to 23)

Table 28: Association of Other Prognostic Markers with EGFR Expression

Patient Characte -ristics	**Lymphatic Invasion (%)**		**Vascular Invasion (%)**		**Perineural Invasion (%)**		**Lymphocytic Response (%)**	
	Absent	*Present*	*Absent*	*Present*	*Absent*	*Present*	*Absent*	*Present*
Negative	2(12.5)	14(87.5)	3(18.7)	13(81.3)	7(43.7)	9(56.3)	11(68.7)	5(31.3)
Positive	2(22.2)	7(77.8)	5(55.6)	4(44.4)	4(44.4)	5(55.6)	5(55.6)	4(44.4)
Fisher's exact test	P Value 0.340		P Value 0.096		P Value 0.988		P Value 0.602	

Among the 50 cases followed up for a mean period of 11.5 months, 53.8% of cases with EGFR over expression were alive. The association between EGFR over expression and survival was not statistically significant. (Table 29 & Chart 24)

Table 29: Association of survival with EGFR expression

Survival of patient	**Alive (%)**	**Dead (%)**	**Total (%)**	**Pearson chi square test**
Negative	25 (67.6)	12 (32.4)	37 (100)	
Positive	7 (53.8)	6 (46.2)	13 (100)	P Value
Total No of Cases	32 (64)	18 (36)	50 (100)	0.156

***Correlation of P53 with Various Clinicopathological Factors*:**

The mean age of patients with positivity for p53 was 52.3 years and that of patients with negativity of p53 was 55.7 years. There was no significant difference in the age of presentation among cases in the two groups. (Table 30 & Chart 11)

Table 30: Association of Age of patient with p53 Expression

P53	**No of cases**	**Mean of age(yrs)**	**Std deviation**	**Mann Whitney test**
Negative	23	55.7	9.58	P Value
Positive	27	52.3	12.67	0.388

Among the cases showing p53 over expression, 51.8% were males and 48.2% were females and the association was not found to be statistically significant. (Table 31 & Chart 12)

Table 31: Association of Gender with P53 Expression

Sex	**Male (%)**	**Female (%)**	**Total (%)**
Negative	15 (65.2)	8 (34.8)	23 (100)
Positive	14 (51.8)	13 (48.2)	27 (100)
Total No. of Cases	29 (58)	21 (42)	50 (100)
Pearson chi square test	P Value 0.340		

On correlating with site, higher number (66.7%) of cases with p53 over expression was from pyloroantral region. The association of site of tumour with p53 expression was not found to be statistically significant. (Table 32 & Chart 13)

Table 32: Association of Site of tumour with p53 Expression

Site	**Eso-Cardia (%)**	**Body (%)**	**Pyloro-antrum (%)**	**Pangastric (%)**	**Total (%)**	**Fisher's exact test**
Negative	3 (13.1)	1(4.3)	17 (73.9)	2 (8.7)	23(100)	P Value 0.691
Positive	6 (22.2)	0	18 (66.7)	3 (11.1)	27(100)	
Total No. of Cases	9 (18)	1(2)	35 (70)	5 (10)	50(100)	

On correlating with gross type, among the 27 cases showing p53 over expression, 66.7% were ulceroproliferative type, 18.5% were ulcerative type, 11.1% were diffuse type and only 3.7% were of nodular type. Association of gross type with p53 expression was not statistically significant. (Table33 & Chart 14)

Table 33: Association of Gross Appearance of Tumour and p53 Expression

Gross type	**Ulcera-tive (%)**	**Nodular (%)**	**Ulcero-prolif -erative (%)**	**Diffuse (%)**	**Total (%)**	**Fisher's exact test**
Negative	3 (13.1)	4 (17.3)	14 (60.9)	2 (8.7)	23(100)	P Value 0.441
Positive	5 (18.5)	1 (3.7)	18 (66.7)	3(11.1)	27(100)	
Total No. of Cases	8 (16)	5 (10)	32 (64)	5 (10)	50(100)	

On correlating the size of tumour with p53 expression, of the 12 cases showing over expression of p53, 75% were less than 5 cm but this association was not statistically significant. (Table 34 & Chart 15)

Table 34: Association of Tumour Size with p53 Expression

Size	<5cm (%)	>=5cm (%)	Total (%)	Fisher's exact test
Negative	10 (76.9)	3 (23.1)	13 (100)	
Positive	9 (75)	3 (25)	12 (100)	P Value
Total No. of Cases	19 (75)	6 (25)	25 (100)	0.678

Among the cases showing p53 over expression, 74.1% were of tubular type, 3.7% were of papillary type. Diffuse type, signet ring cell type and mucinous type constituted for 7.4% of cases each. No statistically significant association between histological type and p53 expression was found. (Table 35 & Chart 16)

Table 35: Association of Histological Type with p53 Expression

Histologi-cal type	Diffuse type (%)	Signet ring cell type (%)	Tubular type (%)	Papillary type (%)	Mucinous type (%)	Total (%)	Fisher's exact test
Negative	7 (30.4)	3 (13.1)	12 (52.1)	0	1 (4.4)	23(100)	
Positive	2 (7.4)	2 (7.4)	20 (74.1)	1 (3.7)	2 (7.4)	27(100)	PValue 0.197
Total No. of Cases	9 (18)	5 (10)	32 (64)	1 (2)	3 (6)	50(100)	

On correlating with Lauren's classification, 77.8% of cases with p53 positivity were of intestinal type and only 22.2% of cases with p53 positivity were of diffuse type and the association was not found to be statistically (Table 36 & Chart 17)

Table 36: Association of Lauren's Classification with p53 Expression

Lauren's	**Intestinal type (%)**	**Diffuse type (%)**	**Total (%)**	**Pearson chi square test**
Negative	12 (52.2)	11 (47.8)	23 (100)	P value 0.057
Positive	21 (77.8)	6 (22.2)	27 (100)	
Total No. of Cases	33 (66)	17 (34)	50 (100)	

Positivity for p53 was comparatively more with moderately differentiated and poorly differentiated grades (37.1% each) than well differentiated grade (25.8%). (Table 37& Chart 18)

Table 37: Association of Tumour Grade with p53 Expression

Grade	**Well Differentiated (%)**	**Moderately Differentiated (%)**	**Poorly Differentiated (%)**	**Total (%)**	**Fisher's exact Test**
Negative	2 (8.8)	7 (30.4)	14 (60.8)	23(100)	P value 0.159
Positive	7 (25.8)	10 (37.1)	10 (37.1)	27(100)	
Total No. of Cases	9 (18)	17 (34)	24 (48)	50(100)	

On correlating with the depth of infiltration for the 25 resected specimens for which p53 expression was studied, 84.6% of cases with p53 positivity showed infiltration up to T3 level and 15.4 % cases showed infiltration up to T2 level none of the cases were showing T4 level of infiltration. Association of depth of infiltration with p 53 expression was not statistically significant.(Table 38 & Chart 19).

Table 38: Association of Depth of Infiltration with p53 Expression

Depth	**T2 (%)**	**T3 (%)**	**T4 (%)**	**Total(%)**	**Fisher's exact test**
Negative	1 (8.3)	8 (66.7)	3 (25)	12 (100)	P value 0.152
Positive	2 (15.4)	11 (84.6)	0	13 (100)	
Total No. of Cases	3 (12)	19 (76)	3 (12)	25 (100)	

Of the 25 resected specimens for which p53 expression was studied, 84.7% cases with p53 positivity showed lymphatic invasion, 61.6% of cases with p53 positivity showed vascular invasion, 53.8% of cases with p53 positivity showed perineural invasion and 30.8% cases with p53 positivity showed lymphocytic response. These associations were not statistically significant. (Table 39 & Charts 20 to 23)

Table 39: Association of Prognostic Markers with p53 Expression

Patient Characte -ristics	Lymphatic Invasion (%)		Vascular Invasion (%)		Perineural Invasion (%)		Lymphocytic Infiltration (%)	
	Absent	*Present*	*Absent*	*Present*	*Absent*	*Present*	*Absent*	*Present*
Negative	2(16.7)	10(83.3)	3(25)	9(75)	5(41.7)	7(58.3)	7(58.3)	5(41.7)
Positive	2(15.3)	11(84.7)	5(38.4)	8(61.6)	6(46.2)	7(53.8)	9(69.2)	4(30.8)
Fisher's exact test	P Value 0.930		P Value 0.471		P Value 0.821		P Value 0.571	

On correlating with survival, no statistically significant association was found between p53 expression and survival of patient. (Table 40 & Chart 24)

Table 40: Association of survival with p53 expression

Survival of patient	Alive (%)	Dead (%)	Total (%)	Pearson chi square test
Negative	16 (69.6)	7 (30.4)	23 (100)	
Positive	16 (59.3)	11 (40.7)	27 (100)	P value
Total No of Cases	32 (64)	18 (36)	50 (100)	0.449

Correlation of HER-2/Neu with Various Clinicopathological Factors:

The mean age of patients with HER-2/Neu over expression was 49.6 years and that of patients without HER-2/Neu over expression was 55.32 years. There was no significant difference in the age at presentation between the two groups (Table 41 & Chart 11)

Table 41: Association of Age of patient with HER-2/Neu Expression

HER-2/Neu	No of cases	Mean of age (yrs)	Std deviation	Mann Whitney test
Negative	40	55.32	11.24	P value 0.155
Positive	10	49.6	11.07	

Of the cases showing HER-2/Neu over expression, 40% were males and 60% were females and there was no significant difference in sex wise distribution. (Table 42 & Chart 12)

Table 42: Association of Gender with HER-2/Neu Expression

Sex	Male (%)	Female (%)	Total (%)
Negative	25 (62.5)	15 (37.5)	40 (100)
Positive	4 (40)	6 (60)	10 (100)
Total No. of Cases	29 (58)	21 (42)	50 (100)
Fisher's exact test	P Value 0.197		

Among the cases showing HER-2/Neu over expression, 60% were from pyloroantral region, 20% were from eso-cardiac region. Only 10% of cases from body and 10% of cases with pangastric involvement showed HER-2/Neu over expression. Association of site of tumour with HER-2/Neu over expression was not statistically significant. (Table43 & Chart 13)

Table 43: Association of Site with HER-2/Neu Expression

Site	Eso-Cardia (%)	Body (%)	Pyloro-antrum (%)	Pangastric (%)	Total (%)	Fisher's exact test
Negative	7(17.5)	0	29 (72.5)	4 (10)	40 (100)	P Value 0.334
Positive	2(20)	1(10)	6 (60)	1 (10)	10 (100)	
Total No. of Cases	9(18)	1(2)	35 (70)	5 (10)	50 (100)	

Among the cases showing HER-2/Neu over expression, 70% were ulceroproliferative type, 20% were ulcerative type, only 10% were diffuse type and none were nodular type. No statistically significant association was found between gross appearance and HER-2/Neu over expression. (Table44 & Chart 14)

Table 44: Association of Gross Appearance with HER-2/Neu Expression

Gross type	Ulcerative (%)	Nodular (%)	Ulcero Proliferative (%)	Diffuse (%)	Total (%)	Fisher's exact test
Negative	6 (15)	5 (12.5)	25 (62.5)	4 (10)	40 (100)	P Value 0.695
Positive	2 (20)	0	7 (70)	1 (10)	10 (20)	
Total No. of Cases	8 (16)	5 (10)	32 (64)	5 (10)	50(100)	

Among the cases showing HER-2/Neu over expression, 75% were less than 5 cm and the association was not statistically significant. (Table 45 & Chart 15)

Table 45: Association of Tumour Size with HER-2/Neu Expression

Size	**<5cm (%)**	**>=5cm (%)**	**Total (%)**	**Fisher's exact test**
Negative	13(76.5)	4(23.5)	17(100)	P Value 0.668
Positive	6(75)	2(25)	8(100)	
Total No. of Cases	19(76)	6(24)	25(100)	

Of the cases showing HER-2/Neu over expression, 80% were tubular type, 10% were papillary type, 10% were mucinous type and none were diffuse or signet ring cell types. No statistically significant association was found between histological type and HER-2/Neu over expression. (Table 46 & Chart 16)

Table 46: Association of Histological Type with HER-2/Neu Expression

Histologi-cal type	**Diffuse type (%)**	**Signet ring cell type (%)**	**Tubular type (%)**	**Papillary type (%)**	**Mucinous type (%)**	**Total (%)**	**Fisher's exact test**
Negative	9 (22.5)	5 (12.5)	24 (60)	0	2 (5)	40(100)	PValue 0.084
Positive	0	0	8 (80)	1 (10)	1 (10)	10(100)	
Total No. of Cases	9 (18)	5 (10)	32 (64)	1 (2)	3 (6)	50(100)	

On correlating with Lauren's classification, 90% of cases with HER-2/Neu over expression were of intestinal type and only 10% of cases with HER-2/Neu over expression were diffuse type but the association was not statistically significant. (Table 47 & Chart 17)

Table 47: Association of Lauren's Classification with HER-2/Neu Expression

Lauren's	Intestinal type (%)	Diffuse type (%)	Total (%)	Pearson chi square test
Negative	24 (60)	16 (40)	40 (100)	P value 0.073
Positive	9 (90)	1 (10)	10 (100)	
Total No. of Cases	33 (66)	17 (34)	50 (100)	

Positivity for HER-2/Neu was seem to be more with poorly differentiated cases (40%) than moderately differentiated (30%) or well differentiated (30%) cases, but the association was not statistically significant. (Table 48 & Chart 18)

Table 48: Association of Tumour Grade with HER-2/Neu Expression

Grade	Well Differentiated (%)	Moderately Differentiated (%)	Poorly Differentiated (%)	Total (%)	Fisher's exact test
Negative	6 (15)	14 (35)	20 (50)	40(100)	P value 0.542
Positive	3 (30)	3 (30)	4 (40)	10(100)	
Total No. of Cases	9 (18)	17 (34)	24 (48)	50(100)	

Among the 25 resected specimens for which HER-2/Neu expression was studied, all the cases with HER-2/Neu over expression showed depth of infiltration up to T3 level but the association was not statistically significant. (Table 49 & Chart 19)

Table 49: Association of Depth of Infiltration with HER-2/Neu Expression

Depth	**T2 (%)**	**T3 (%)**	**T4 (%)**	**Total (%)**	**Fisher's exact test**
Negative	3 (15.8)	13 (68.4)	3 (15.8)	19 (100)	P value 0.287
Positive	0	6 (100)	0	6 (100)	
Total No. of Cases	3 (12)	19 (76)	3 (12)	25 (100)	

100% of cases with HER-2/Neu over expression showed lymphatic invasion, 66.7% of cases with HER-2/Neu over expression showed vascular invasion and perineural invasion, only 16.7% of cases with HER-2/Neu over expression showed lymphocytic response. Of these, only the association of lymphocytic response to HER-2/Neu over expression was statistically significant. (Table 50 & Charts 20 to 23)

Table 50: Association of Prognostic Markers with HER-2/Neu Expression

Patient Characte-ristics	Lymphatic Invasion (%)		Vascular Invasion (%)		Perineural Invasion (%)		Lymphocytic Response (%)	
	Absent	*Present*	*Absent*	*Present*	*Absent*	*Present*	*Absent*	*Present*
Negative	4 (21.5)	15(78.5)	6(31.6)	13(68.4)	9(47.4)	10(52.6)	11(57.9)	8(42.1)
Positive	0	6(100)	2(33.3)	4(66.7)	2(33.3)	4(66.7)	5(83.3)	1(16.7)
Fisher's exact test	P Value 0.444		P Value 0.425		P Value 0.763		**P Value 0.003**	

Among the 50 cases followed up for a mean period of 11.5 months, no statistically significant association was found between HER-2/Neu expression and survival. (Table 51 & Chart 24)

Table 51: Association of survival with HER-2/Neu

Survival of patient	Alive (%)	Dead (%)	Total (%)	Fisher's exact test
Negative	24 (60)	16 (40)	40 (100)	P Value 0.201
Positive	8 (80)	2 (20)	10 (100)	
Total No. of Cases	32 (64)	18 (36)	50 (100)	

On comparing the expression of E Cadherin, EGFR, p53 and HER-2/Neu with each other, reduced expression of E Cadherin was found to be significantly associated with EGFR over expression and HER-2/Neu over expression. (Table 52)

Table 52: Comparison of expressions of E Cadherin with EGFR, p53 and Her2/Neu:

	E Cadherin	**EGFR**	**p53**	**HER-2/ Neu**
E Cadherin	K=1	K=0.2201**	K=0.2101	K=0.4248**
EGFR	K=0.2201**	K= 1	K= -0.0786	K= 0.1573
p53	K=0.2101	K= -0.0786	K= 1	K= 0.1221
Her2/ Neu	K=0.4248**	K= 0.1573	K= 0.1221	K= 1

K- Kappa value, **-obtained significant P Value

Figure 7: Gastric Carcinoma- Ulcerative

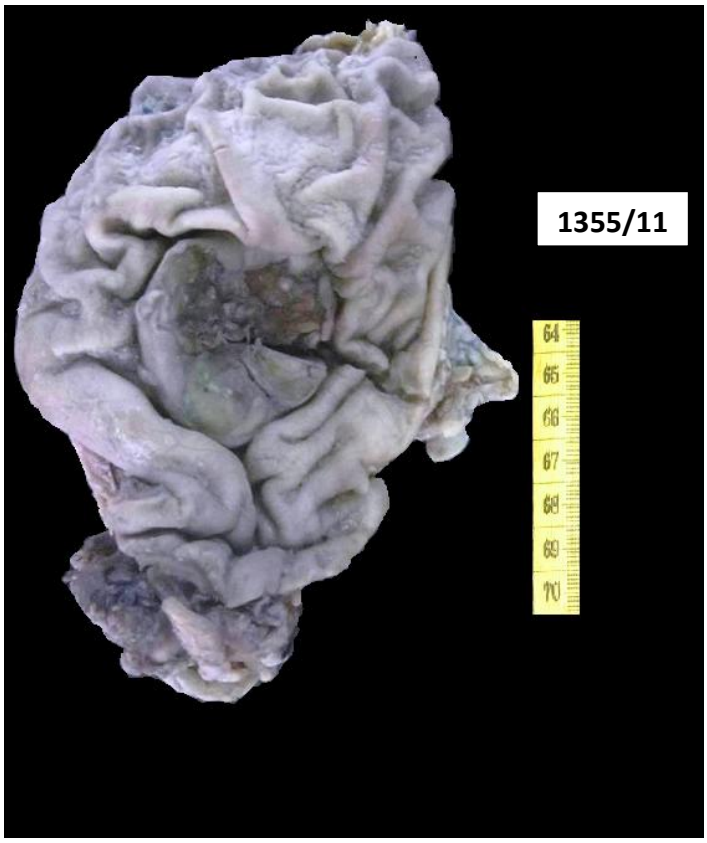


Figure 8: Gastric Carcinoma- Proliferative

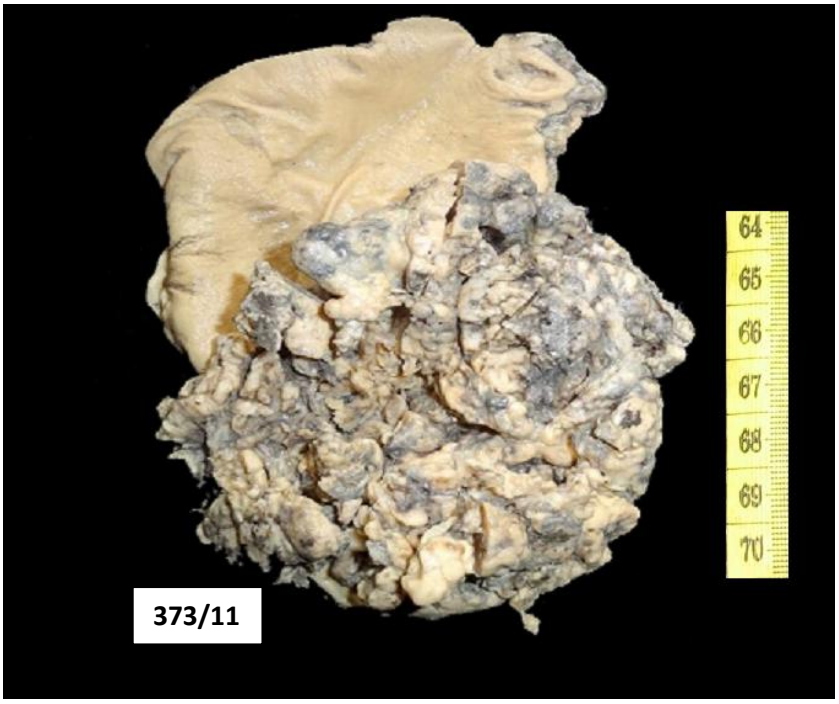


Figure 9: Gastric Carcinoma- Ulceroproliferative

Figure 10: Gastric Carcinoma- Linitis Plastica

Figure 11: Gastric Carcinoma- Mucinous Type

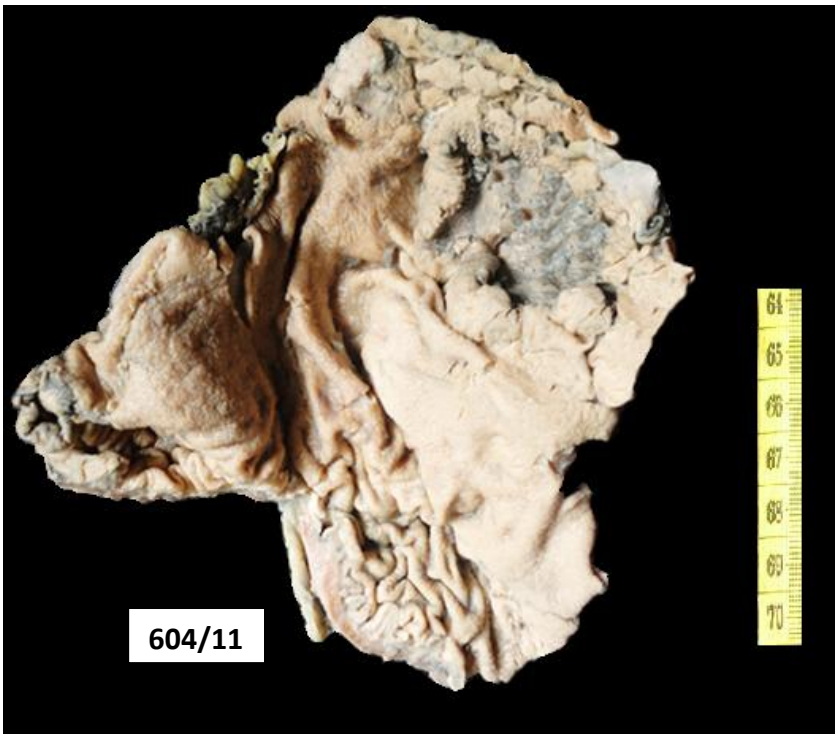


Figure 12: Gastric Carcinoma- Papillary type

Gastric adenocarcinoma- Different grades

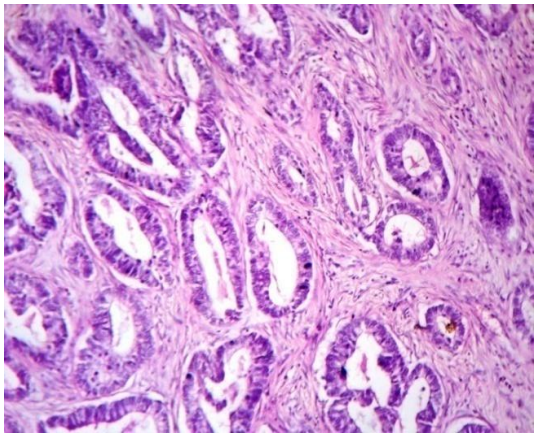


Figure 13: Well differentiated grade showing well formed glands lined by malignant cells, 40x, HPE- 74/11

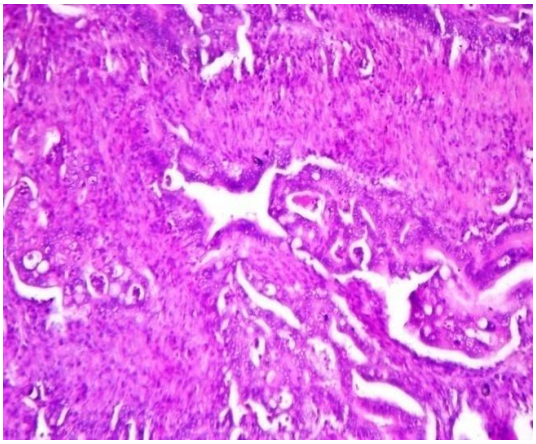


Figure14: Moderately differentiated grade showing cells arranged in groups and glands, 40x, HPE-2048/11

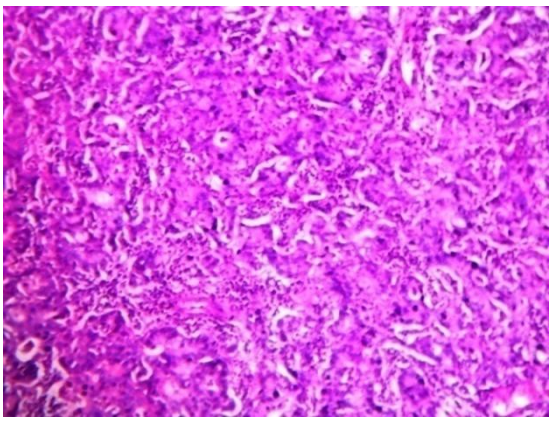


Figure15: Poorly differentiated grade with cells arranged in sheets, 40x, HPE-3914/11

Lauren's Classification

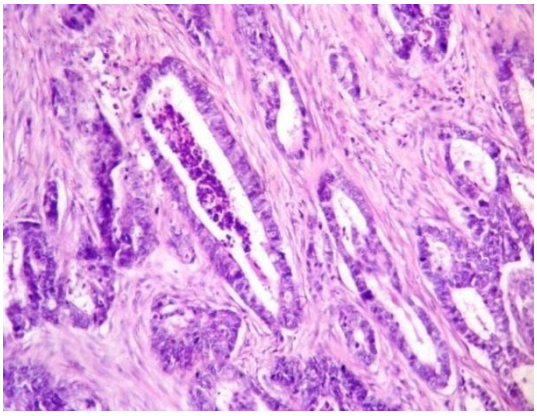


Figure 16: Intestinal type. Tumour cell arranged in glandular pattern, 40x, HPE- 2048/11

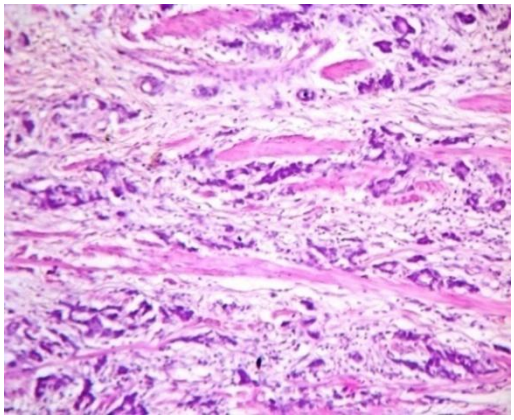


Figure17: Diffuse type. Single cells & small clusters of cells infiltrating muscular layer, 10x, HPE- 2259/11

WHO Classification-Tubular Carcinoma

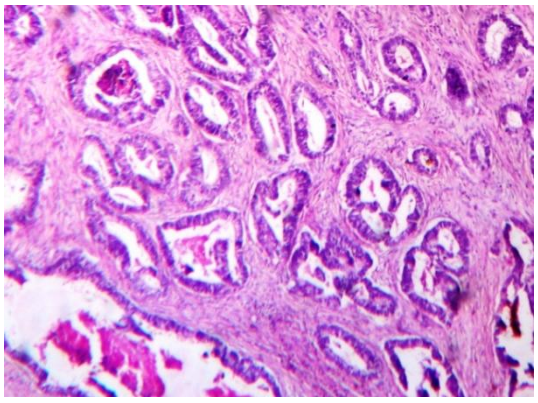


Figure 18: Irregular dilated & branching tubules of varying sizes, 10x, HPE- 5019/11

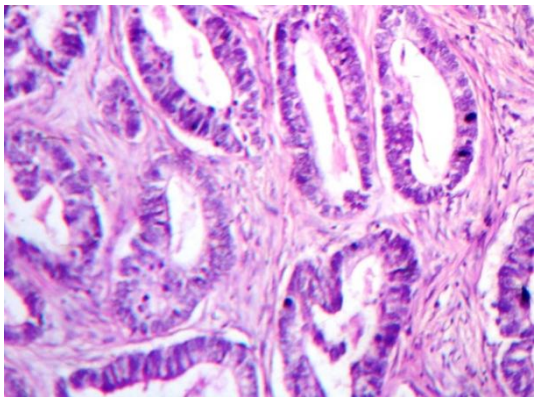


Figure 19: Tubules lined by columnar cells showing atypia and mitosis, 40x, HPE- 5019/11

WHO Classification- Papillary carcinoma

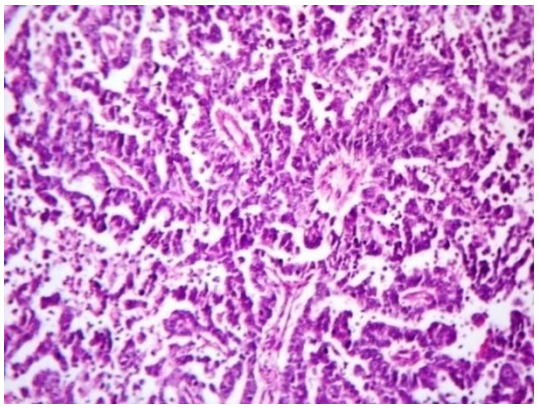


Figure 20: Cross sections of papillae with central fibrovascular core, 40x, HPE-604/11

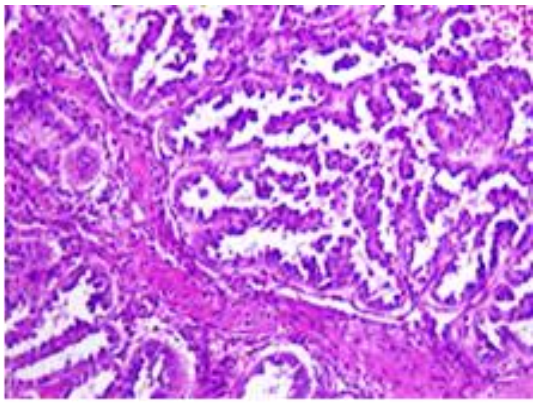


Figure 21: Thin papillae with central fibrovascular core lined by malignant cells, 40x, HPE- 604/11

WHO Classification- Mucinous carcinoma

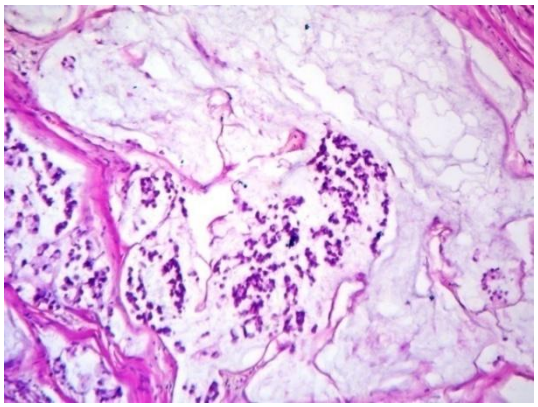


Figure 22: Clusters of cells floating in extracellular mucin, 10x, HPE- 196/11

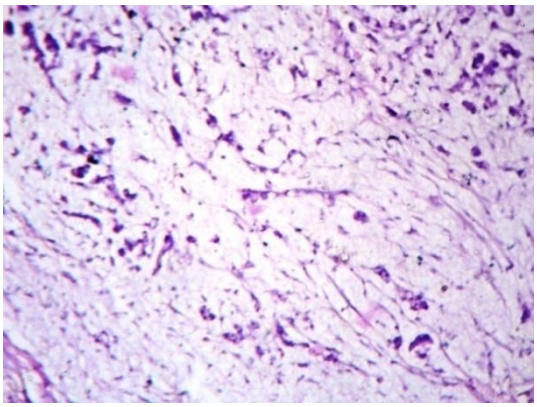


Figure 23: Singly dispersed cells floating in abundant extracellular mucin, 10x, HPE-372/11

WHO Classification- Signet ring cell carcinoma

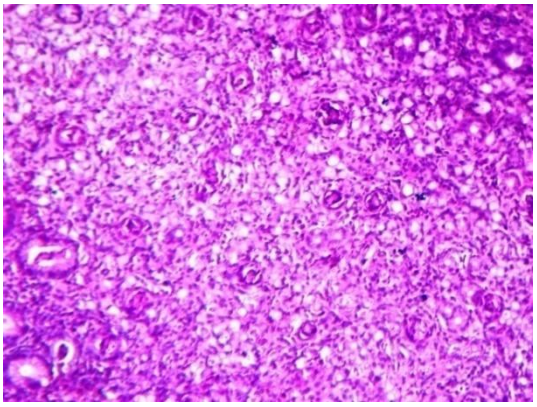


Figure 24: Sheets of diffusely infiltrating signet ring cells, 10x, HPE- 373/11

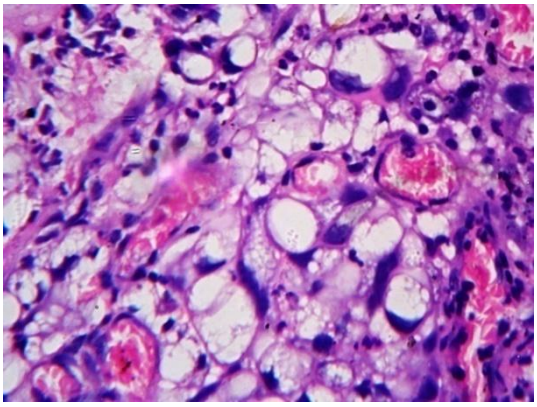


Figure 25: Signet ring cells with intracellular mucin and peripherally pushed nuclei, 40x, HPE- 373/11

WHO Classification- Diffuse carcinoma

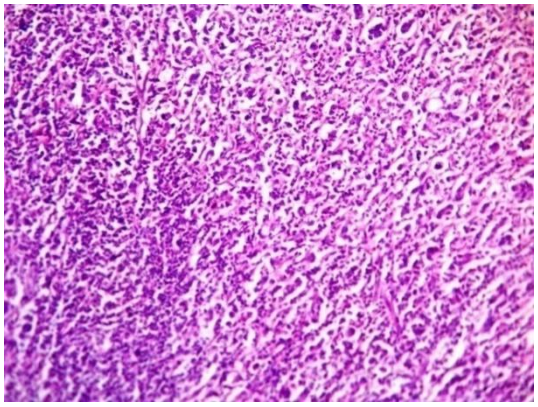


Figure 26: Diffusely infiltrating malignant cells, 10x, HPE-4850/11

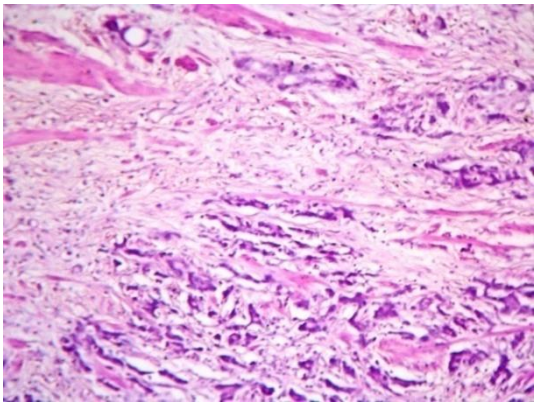


Figure 27: Small clusters and single cells diffusely infiltrating muscular layer, 10x, HPE-2259/11

Other Prognostic Factors

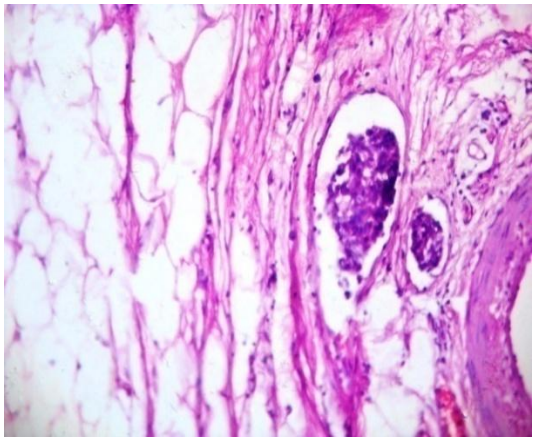


Figure 28: Lymphatic invasion 40x, HPE- 352/11

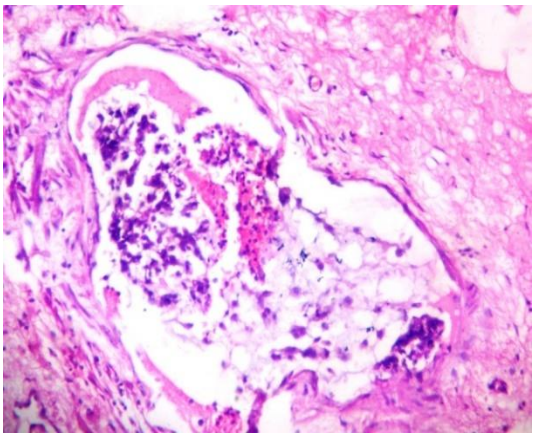


Figure 29: Vascular Invasion, 40x, HPE- 5016/11

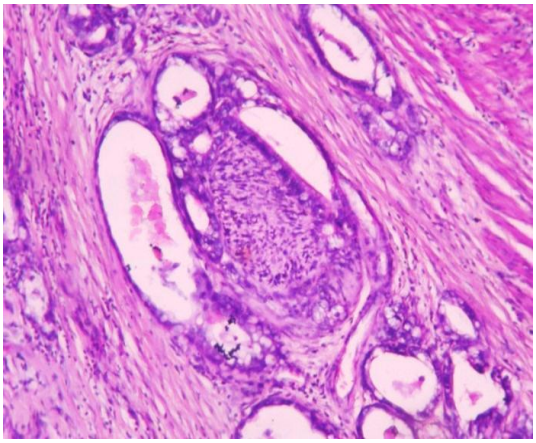


Figure 30: Perineural Invasion, 40x, HPE- 6411/11

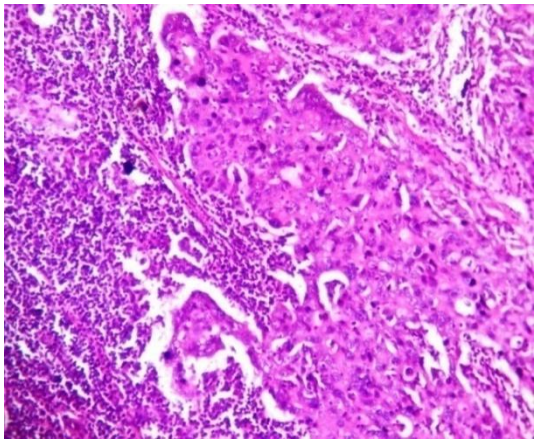


Figure 31: Lymphocytic Response, 40x, HPE- 2836/11

Immunohistochemical Analysis of E Cadherin in Gastric Carcinoma

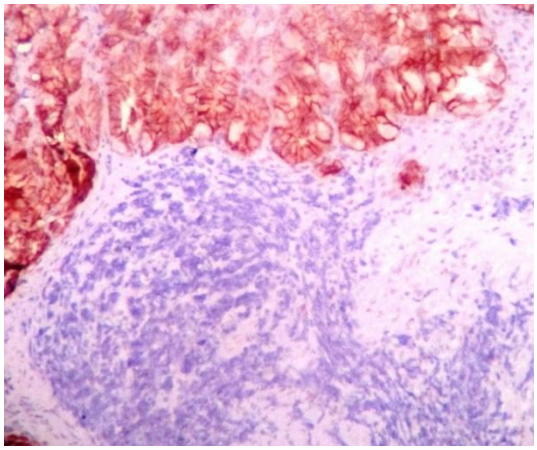


Figure 32: E Cadherin Score 0.Negative staining in tumour cells (Adjacent foci with normal glands show intense membranous positivity),10x, HPE-5631/11

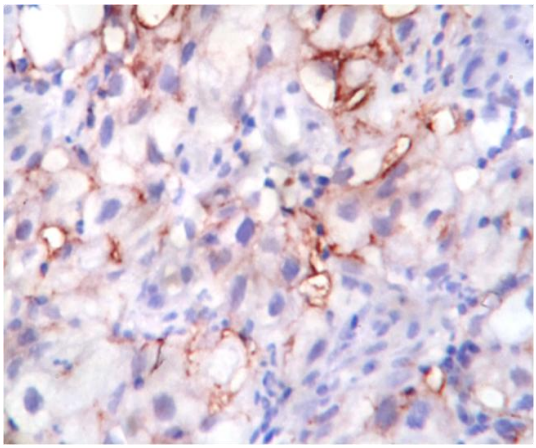


Figure 33:E Cadherin Score 1+, Incomplete membranous staining in less than 20% cells, 40x, HPE- 8896/11

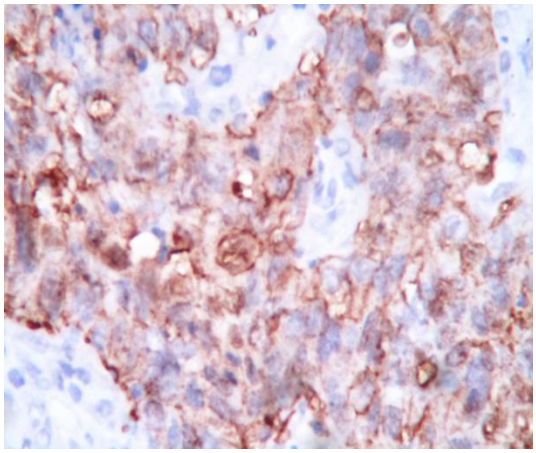


Figure 34: E Cadherin Score 2+, moderate intense complete membranous staining in 60% of tumour cells, 40x, HPE- 8270/11

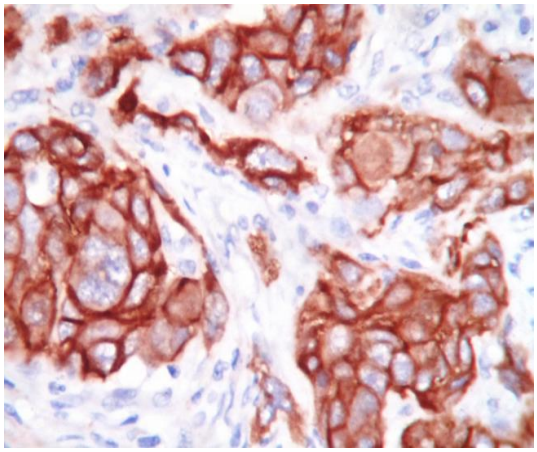


Figure 35: E Cadherin Score 3+, strong intense complete membranous staining in all tumour cells, 40x, HPE- 1306/11

Immunohistochemical Analysis of EGFR in Gastric Carcinoma

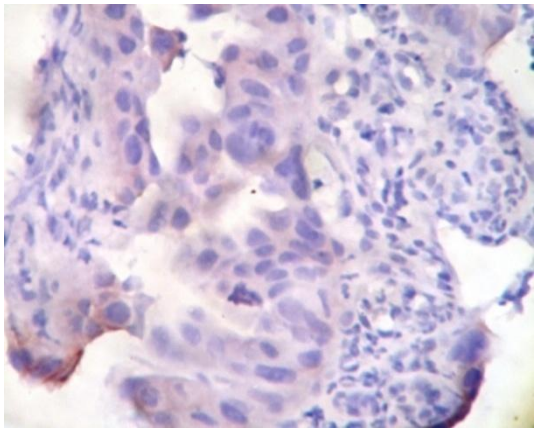


Figure 36: EGFR Score 0, No staining in tumour cells, 40x, HPE- 1658/11

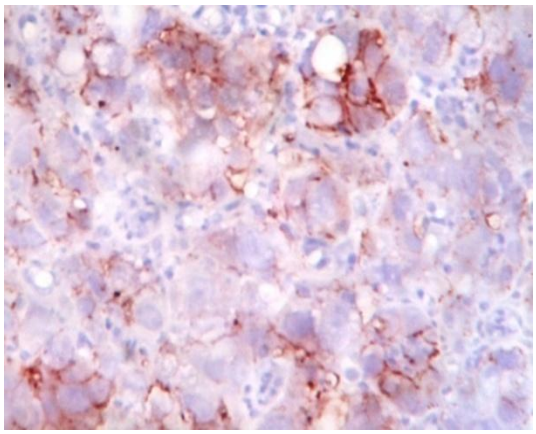


Figure 37: EGFR Score 1+, Incomplete membranous staining in 40% of tumour cells, 40x, HPE- 628/11

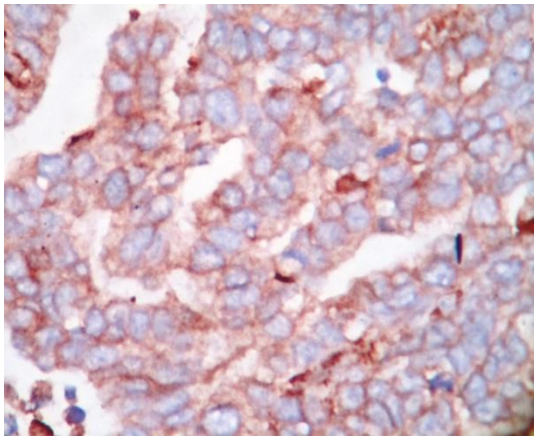


Figure 38: EGFR Score 2+, moderately intense complete membranous staining in all tumour cells, 40x, HPE- 5384/11

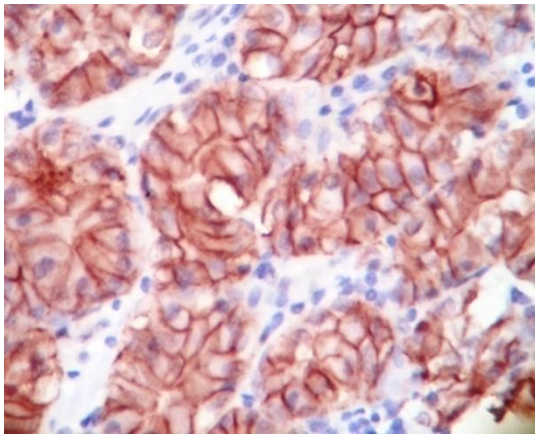


Figure 39: EGFR Score 3+, Complete strong intense membranous staining in all tumour cells, 40x, HPE- 7965/11

Immunohistochemical Analysis of p53 in Gastric Carcinoma

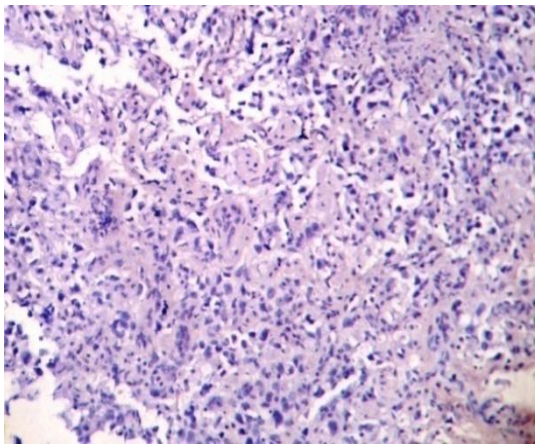


Figure 40: p53 Negative, No staining in all tumour cells, 10x, HPE- 763/11

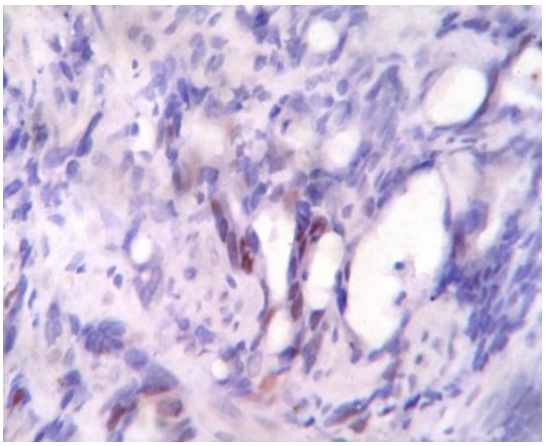


Figure 41: p53 Negative, very weak nuclear staining in < 5% of tumour cells, 40x, HPE- 1382/11

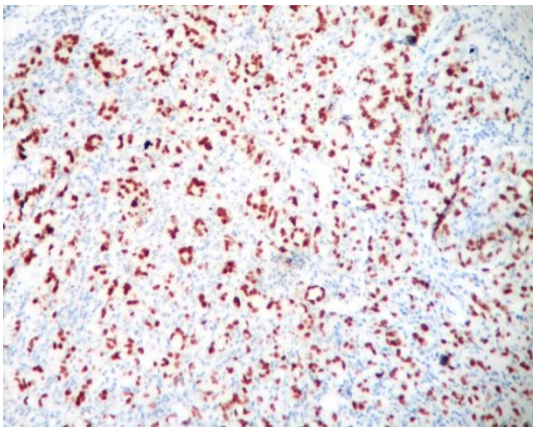


Figure 42: p53 Positive, Strong intense nuclear staining in > 90% tumour cells, 10x, HPE- 1279/11

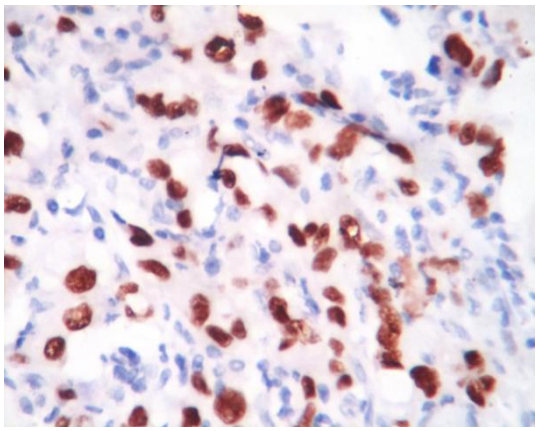


Figure 43: p53 Positive, Strong intense nuclear staining in > 95% of tumour cells, 40x, HPE- 1355/11

Immunohistochemical Analysis of HER-2/Neu in Gastric Carcinoma

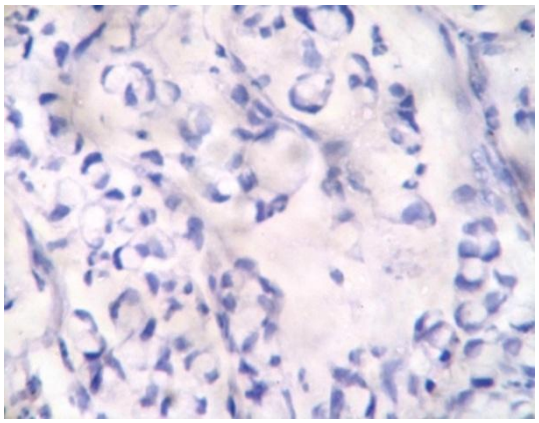


Figure 44: HER-2/Neu Score 0, No staining in all tumour cells, 40x, HPE- 1126/11

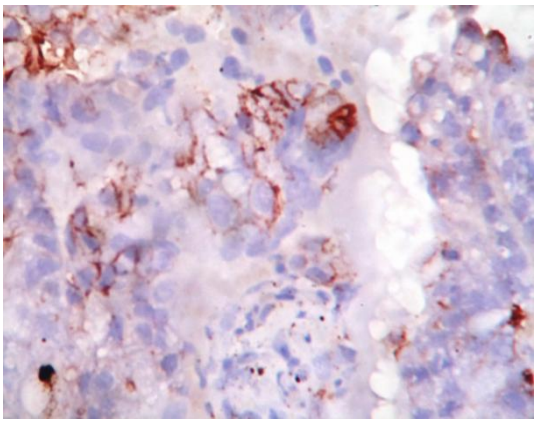


Figure 45: HER-2/Neu Score 1+, Incomplete membranous staining in 40% of tumour cells, 40x, HPE- 3073/11

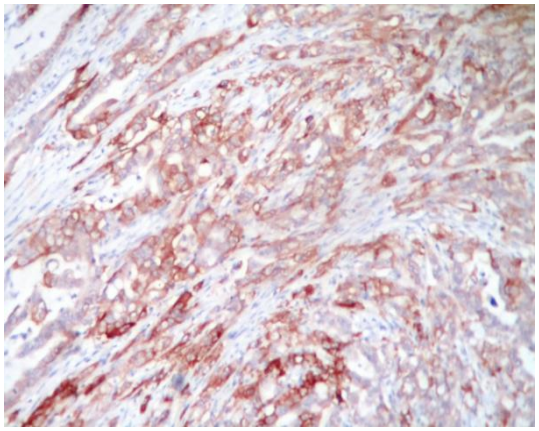


Figure 46: HER-2/Neu Score 2+, Moderate intense complete staining in 90% of tumour cells, 10x, HPE-576/11

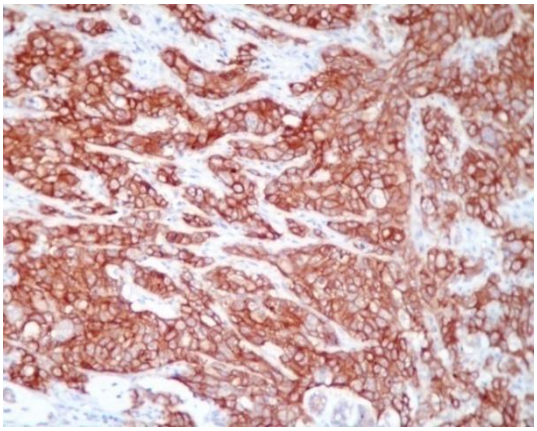


Figure 47: HER-2/Neu Score 3+, Strong intense complete membranous staining in all tumour cells, 40x, HPE- 763/11

FIGURE 48: IMMUNOHISTOCHEMISTRY EQUIPMENT AND KIT

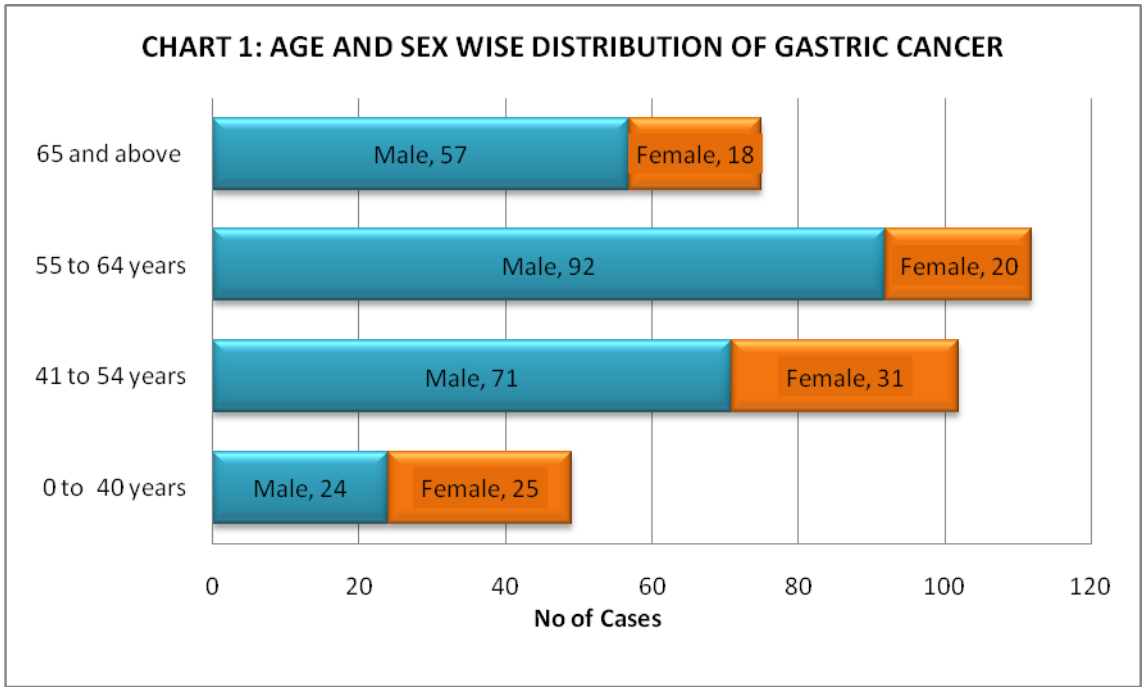


CHART 1: AGE AND SEX WISE DISTRIBUTION OF GASTRIC CANCER
65 and above
Male, 57
Female, 18
55 to 64 years
Male, 92
Female, 20
41 to 54 years
Male, 71
Female, 31
0 to 40 years
Male, 24
Female, 25
0
20
40
60
80
100
120
No of Cases

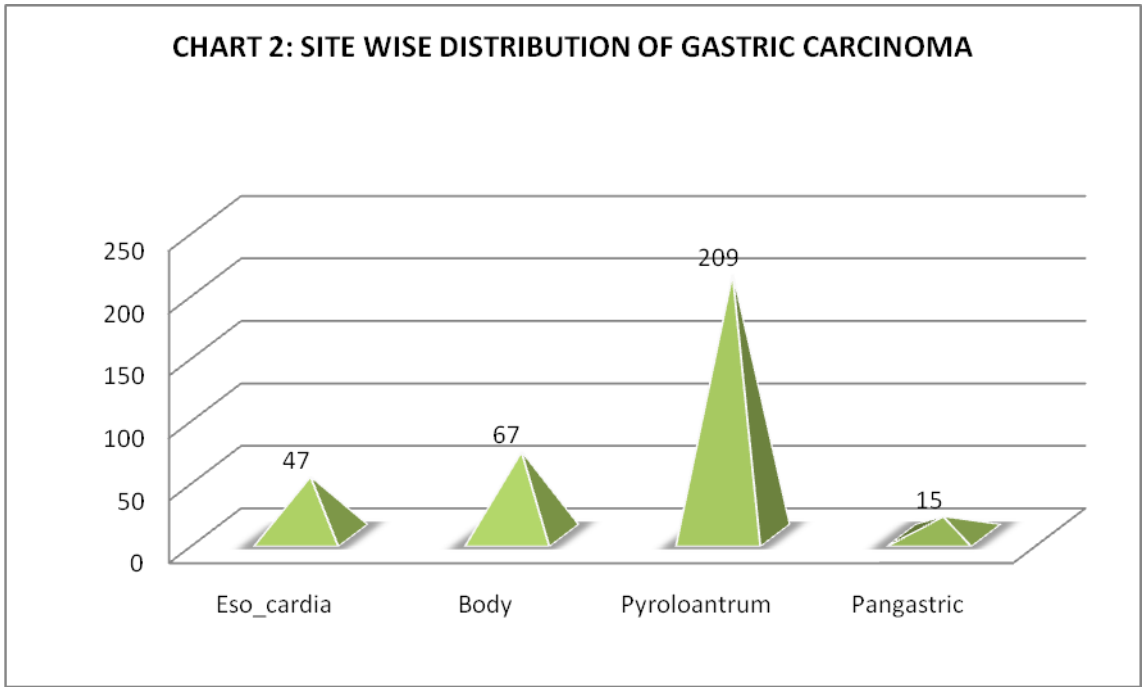


CHART 2: SITE WISE DISTRIBUTION OF GASTRIC CARCINOMA
250
200
150
100
50
0
47
67
209
15
Eso_cardia
Body
Pyroloantrum
Pangastric

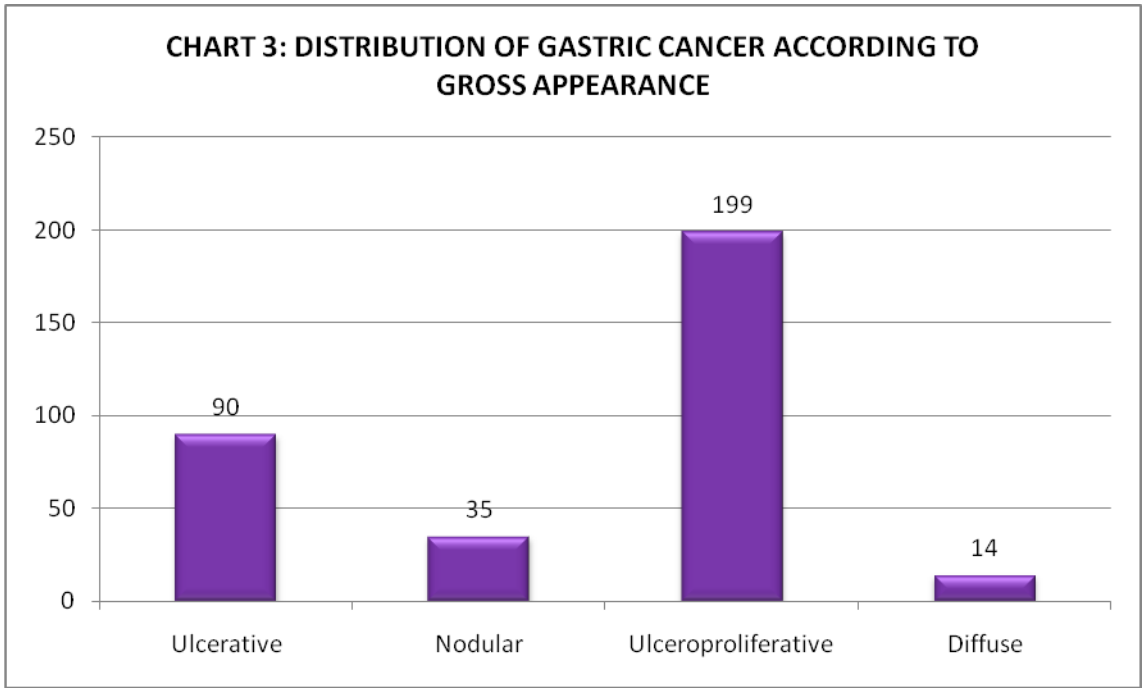


CHART 3: DISTRIBUTION OF GASTRIC CANCER ACCORDING TO GROSS APPEARANCE
250
200
150
100
50
0
90
35
199
14
Ulcerative
Nodular
Ulceroproliferative
Diffuse

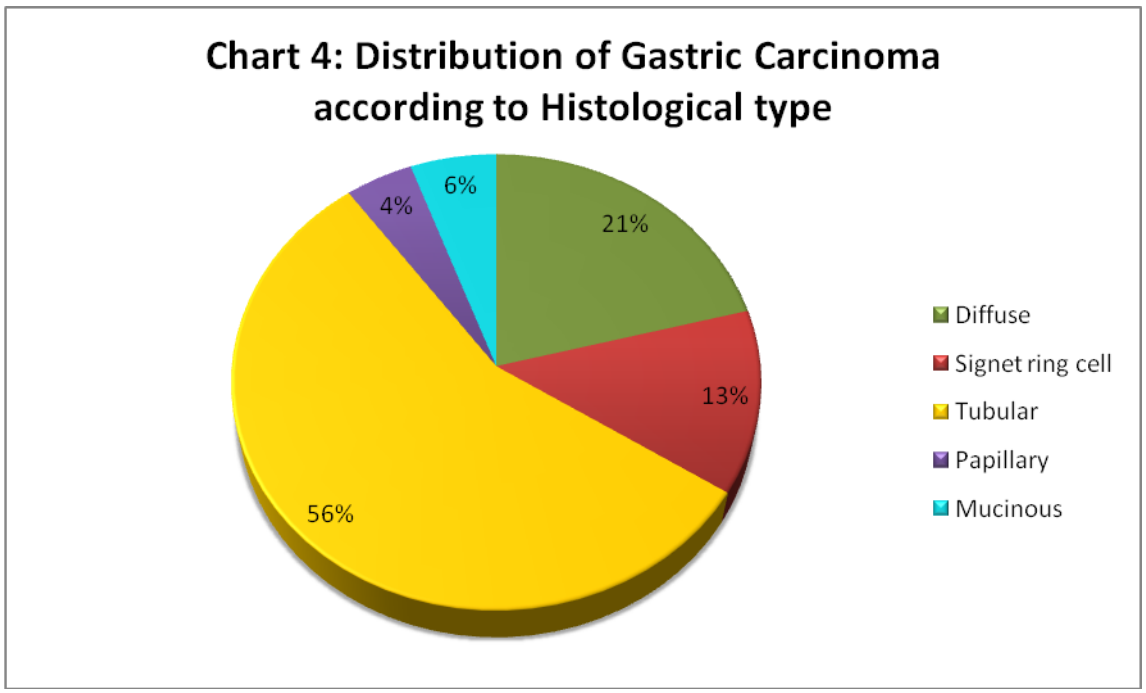


Chart 4: Distribution of Gastric Carcinoma according to Histological type
21%
13%
56%
4%
6%
Diffuse
Signet ring cell
Tubular
Papillary
Mucinous

CHART 5: DISTRIBUTION OF GASTRIC CARCINOMA ACCORDING TO LAUREN'S CLASSIFICATION

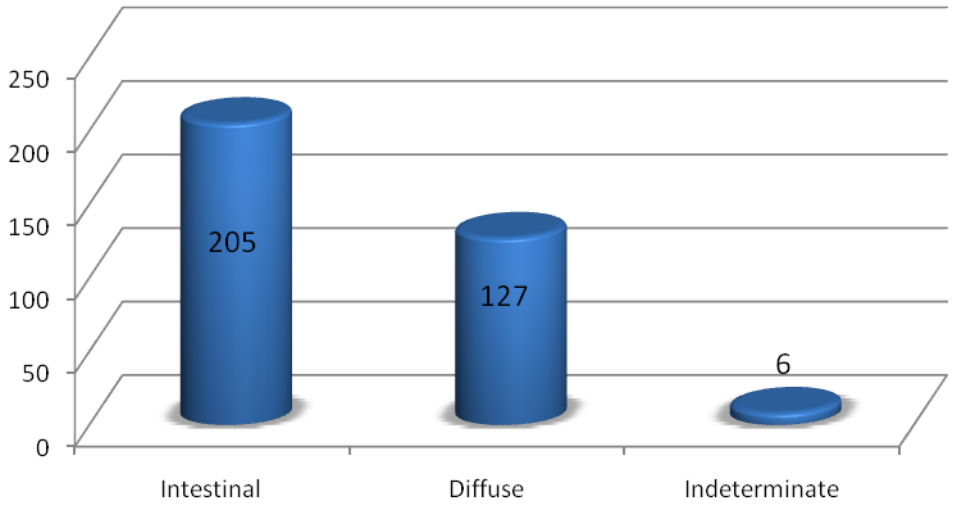
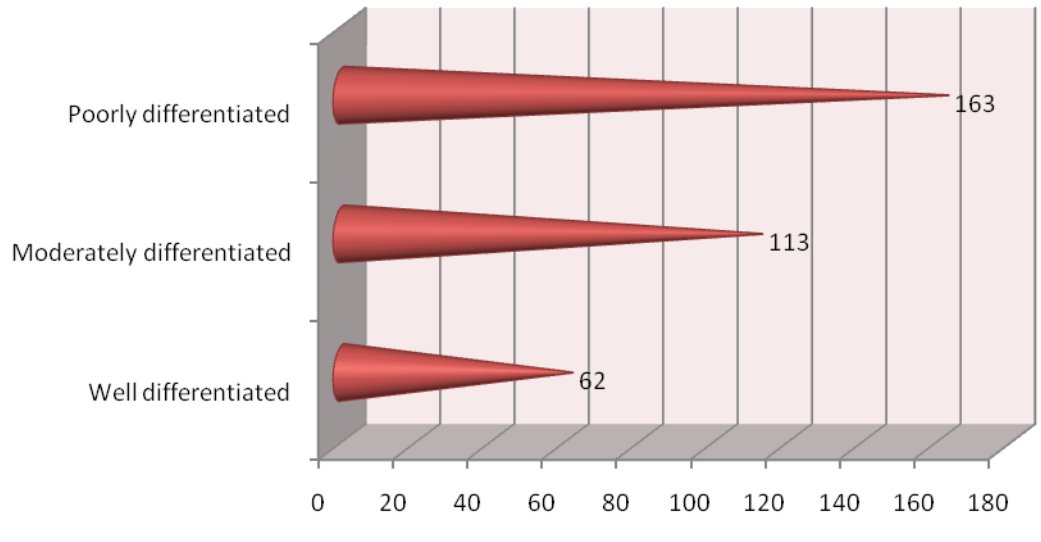


CHART 6: DISTRIBUTION OF GASTRIC CARCINOMA ACCORDING TO HISTOLOGICAL GRADE



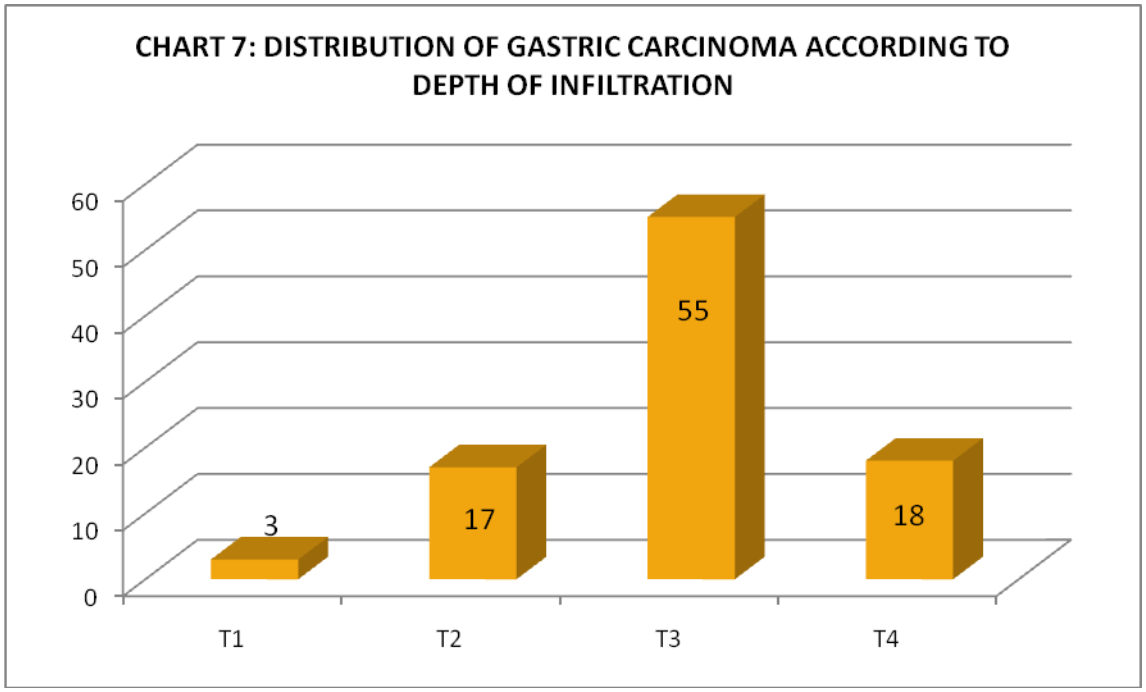


CHART 7: DISTRIBUTION OF GASTRIC CARCINOMA ACCORDING TO DEPTH OF INFILTRATION
60
50
40
30
20
10
0
3
17
55
18
T1
T2
T3
T4

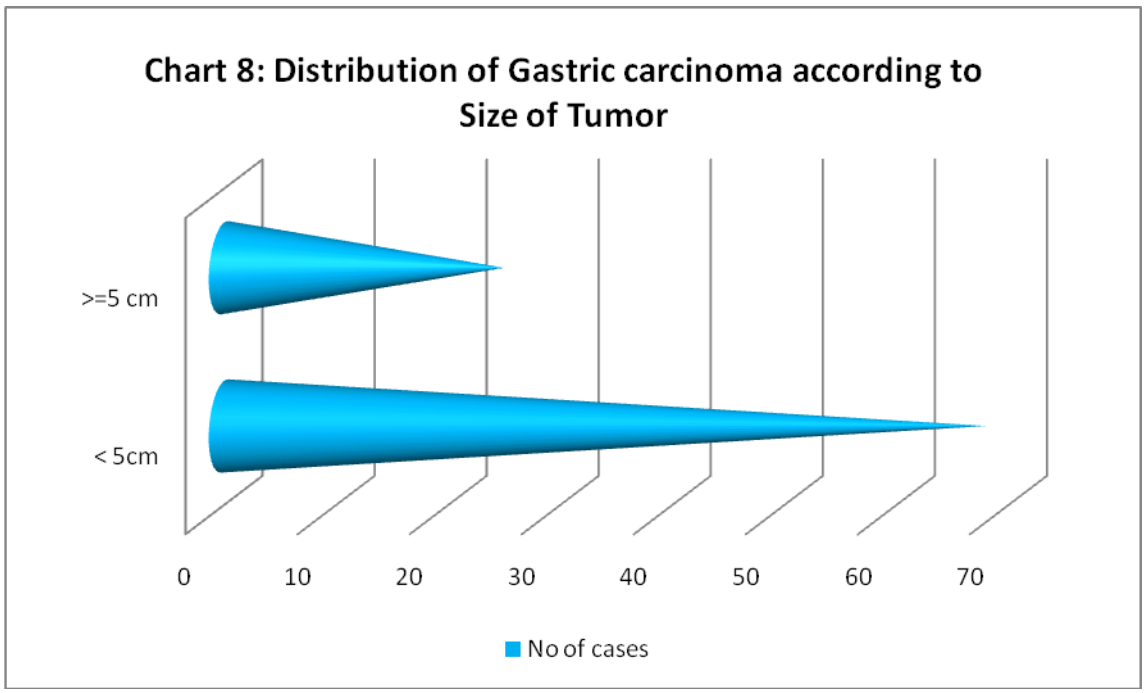


Chart 8: Distribution of Gastric carcinoma according to Size of Tumor
>=5 cm
< 5cm
0
10
20
30
40
50
60
70
No of cases

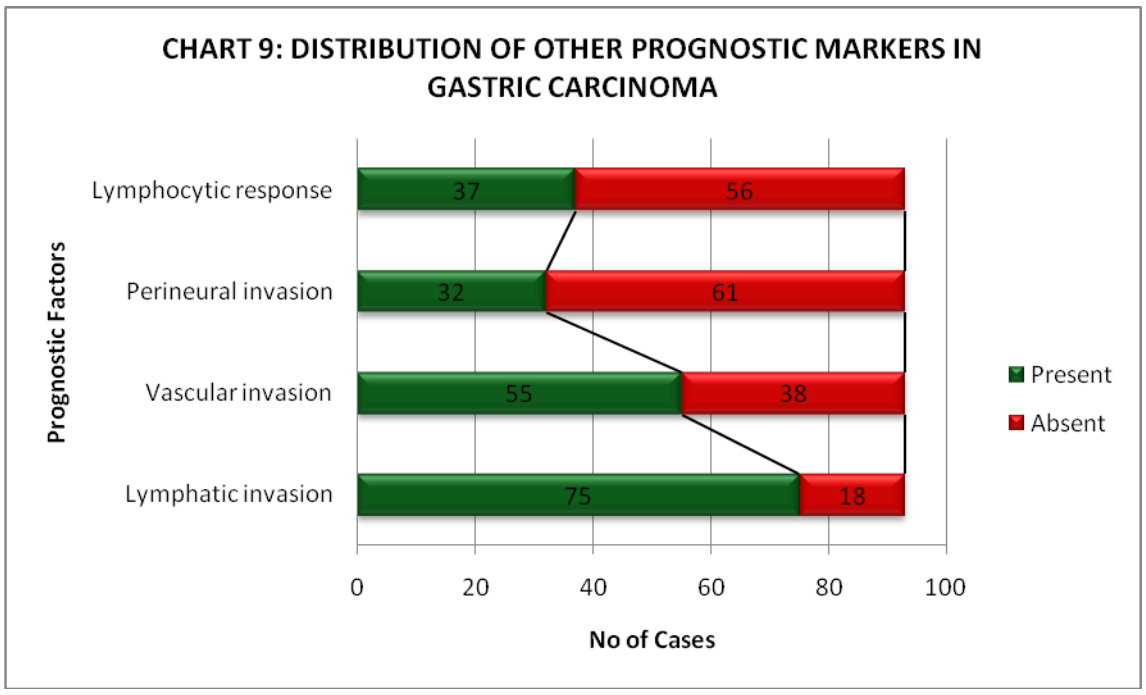


CHART 9: DISTRIBUTION OF OTHER PROGNOSTIC MARKERS IN GASTRIC CARCINOMA
Prognostic Factors
Lymphocytic response
Perineural invasion
Vascular invasion
Lymphatic invasion
37
56
32
61
55
38
75
18
0
20
40
60
80
100
No of Cases
Present
Absent

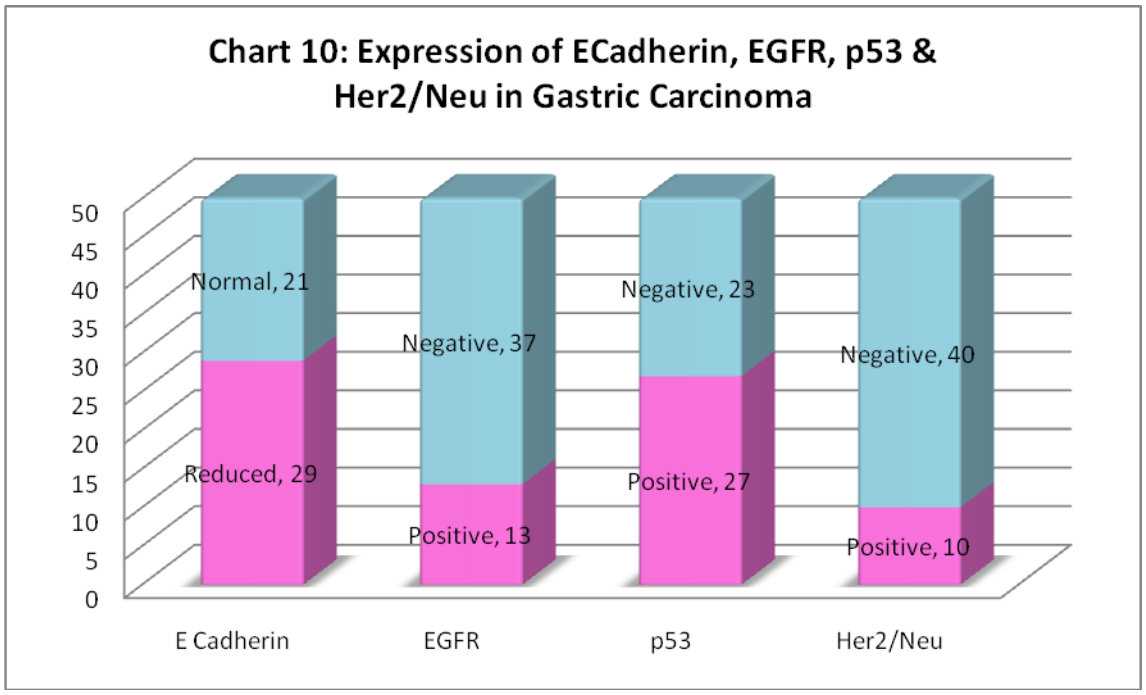


Chart 10: Expression of ECadherin, EGFR, p53 & Her2/Neu in Gastric Carcinoma
50
45
40
35
30
25
20
15
10
5
0
Normal, 21
Reduced, 29
Negative, 37
Positive, 13
Negative, 23
Positive, 27
Negative, 40
Positive, 10
E Cadherin
EGFR
p53
Her2/Neu

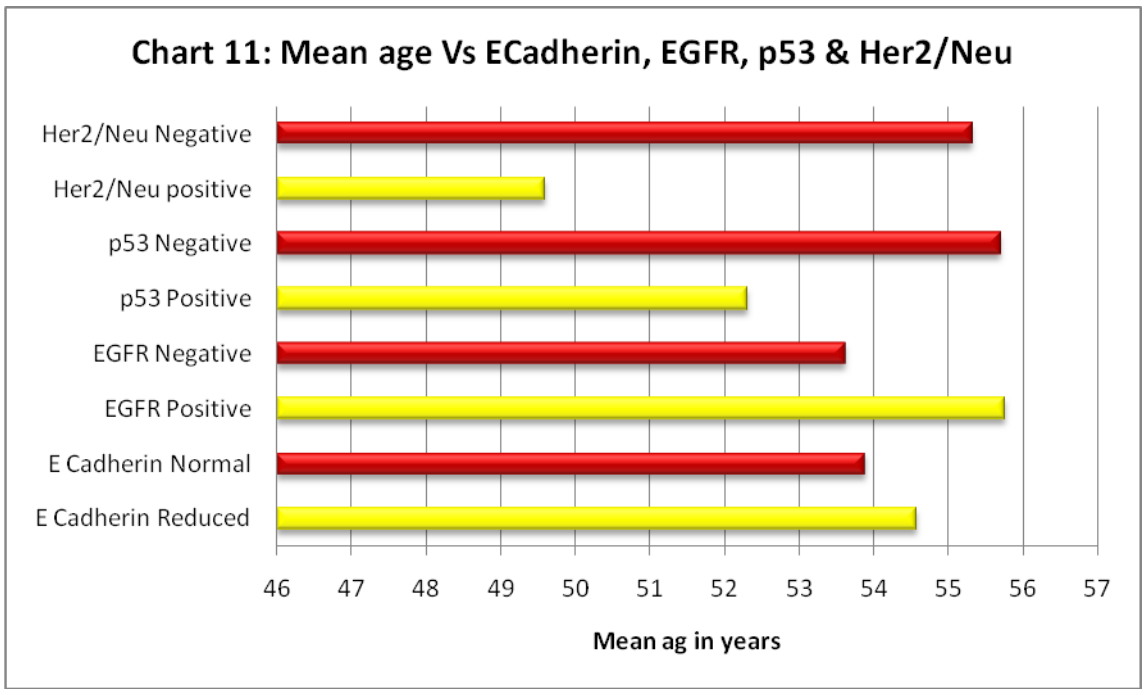


Chart 11: Mean age Vs ECadherin, EGFR, p53 & Her2/Neu
Her2/Neu Negative
Her2/Neu positive
p53 Negative
p53 Positive
EGFR Negative
EGFR Positive
E Cadherin Normal
E Cadherin Reduced
46
47
48
49
50
51
52
53
54
55
56
57
Mean ag in years

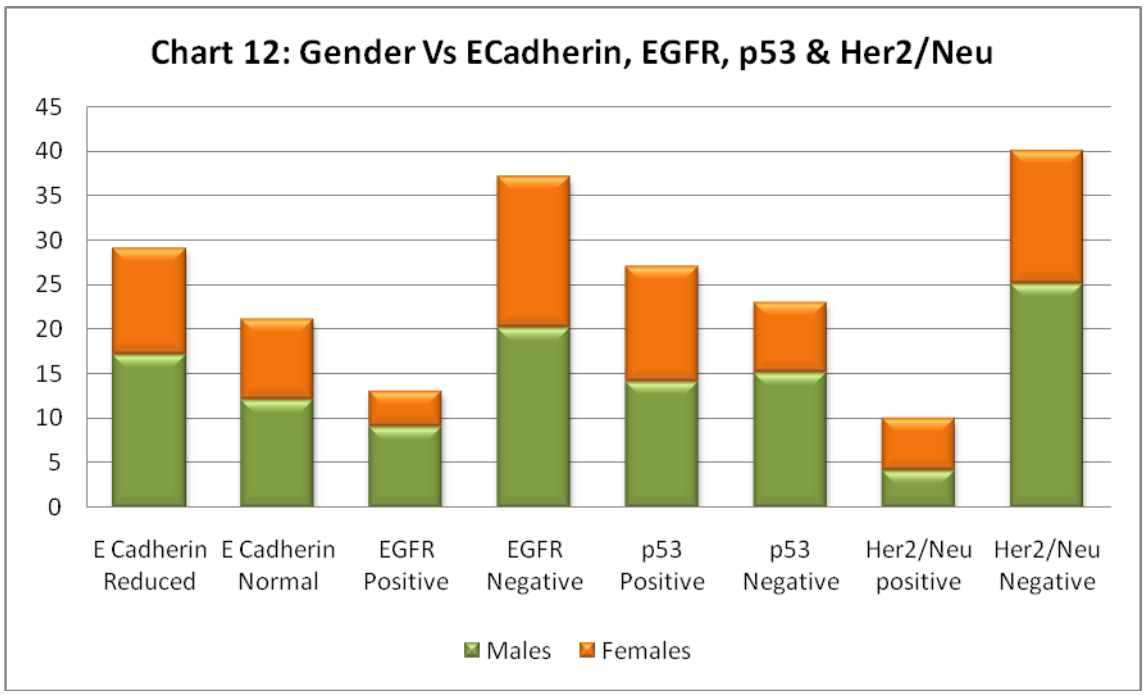
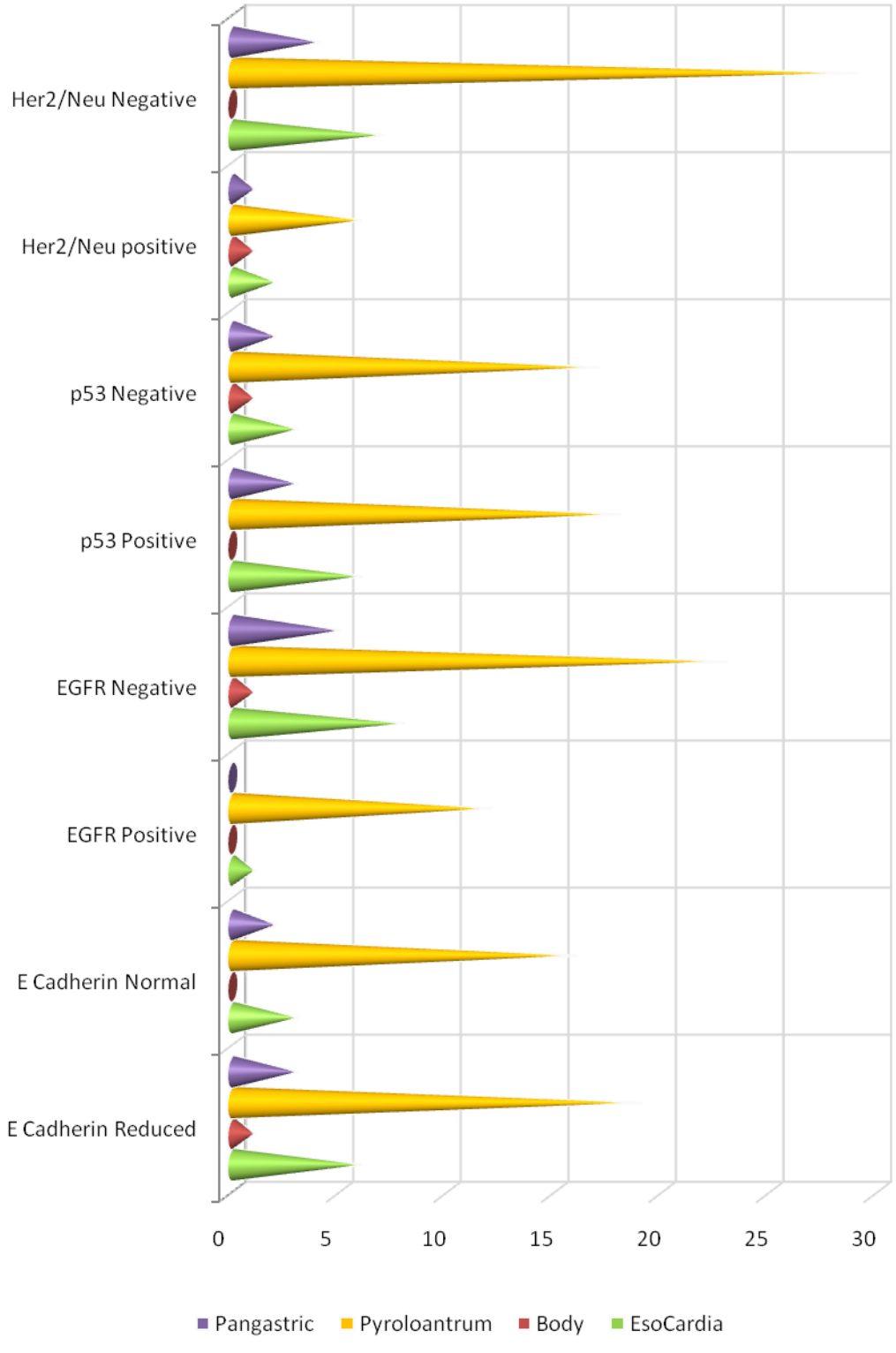


Chart 12: Gender Vs ECadherin, EGFR, p53 & Her2/Neu
45
40
35
30
25
20
15
10
5
0
E Cadherin Reduced
E Cadherin Normal
EGFR Positive
EGFR Negative
p53 Positive
p53 Negative
Her2/Neu positive
Her2/Neu Negative
Males
Females

Chart 13: Site of Gastric Carcinoma Vs ECadherin, EGFR, p53 & Her2/Neu



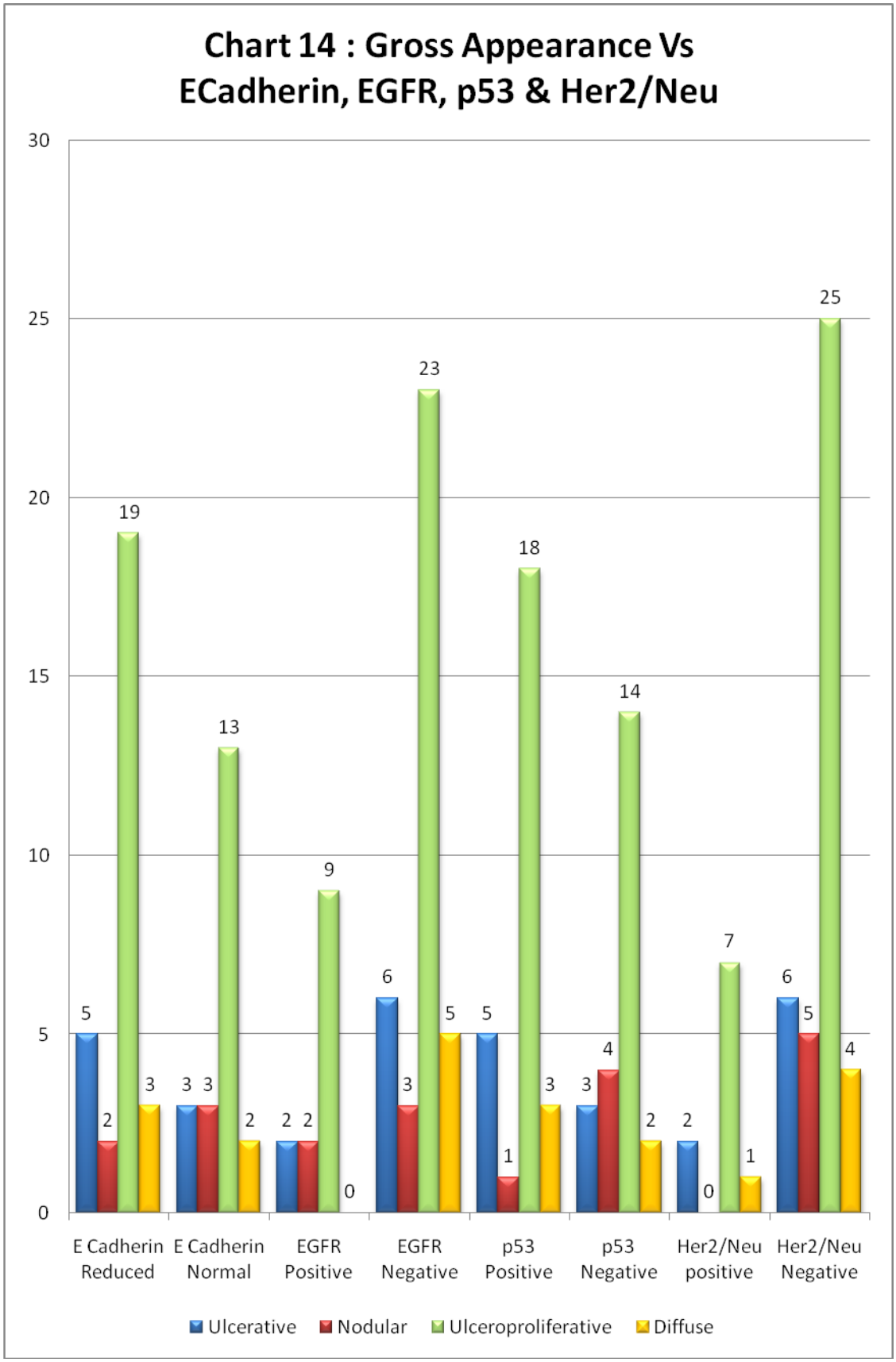


Chart 14 : Gross Appearance Vs ECadherin, EGFR, p53 & Her2/Neu
30
25
20
15
10
5
0
5
2
19
3
3
3
13
2
2
2
9
0
6
3
23
5
5
1
18
3
3
4
14
2
2
0
7
1
6
5
25
4
E Cadherin Reduced
E Cadherin Normal
EGFR Positive
EGFR Negative
p53 Positive
p53 Negative
Her2/Neu positive
Her2/Neu Negative
Ulcerative
Nodular
Ulceroproliferative
Diffuse

Chart 15 : Size Vs ECadherin, EGFR, p53 & Her2/Neu

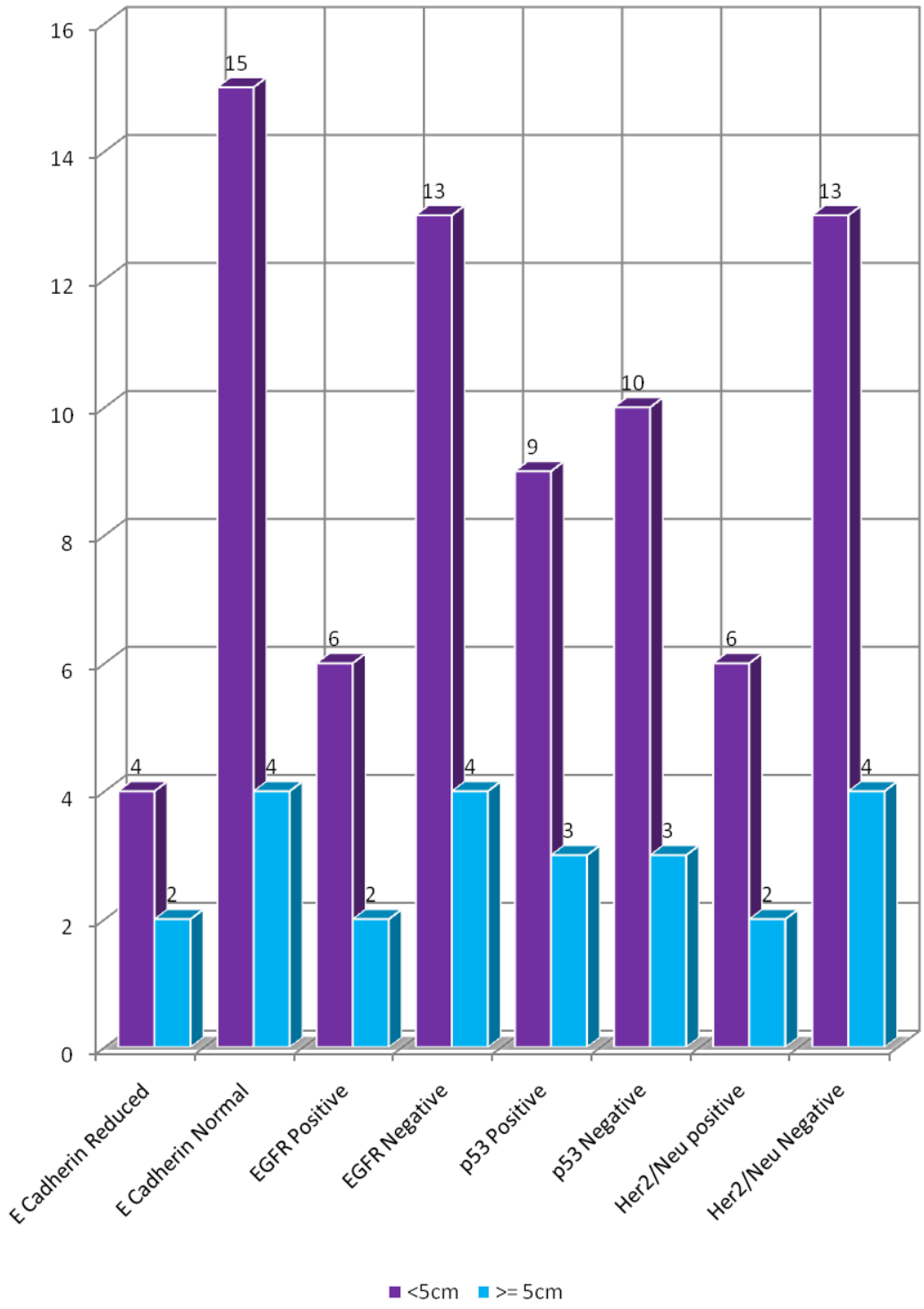
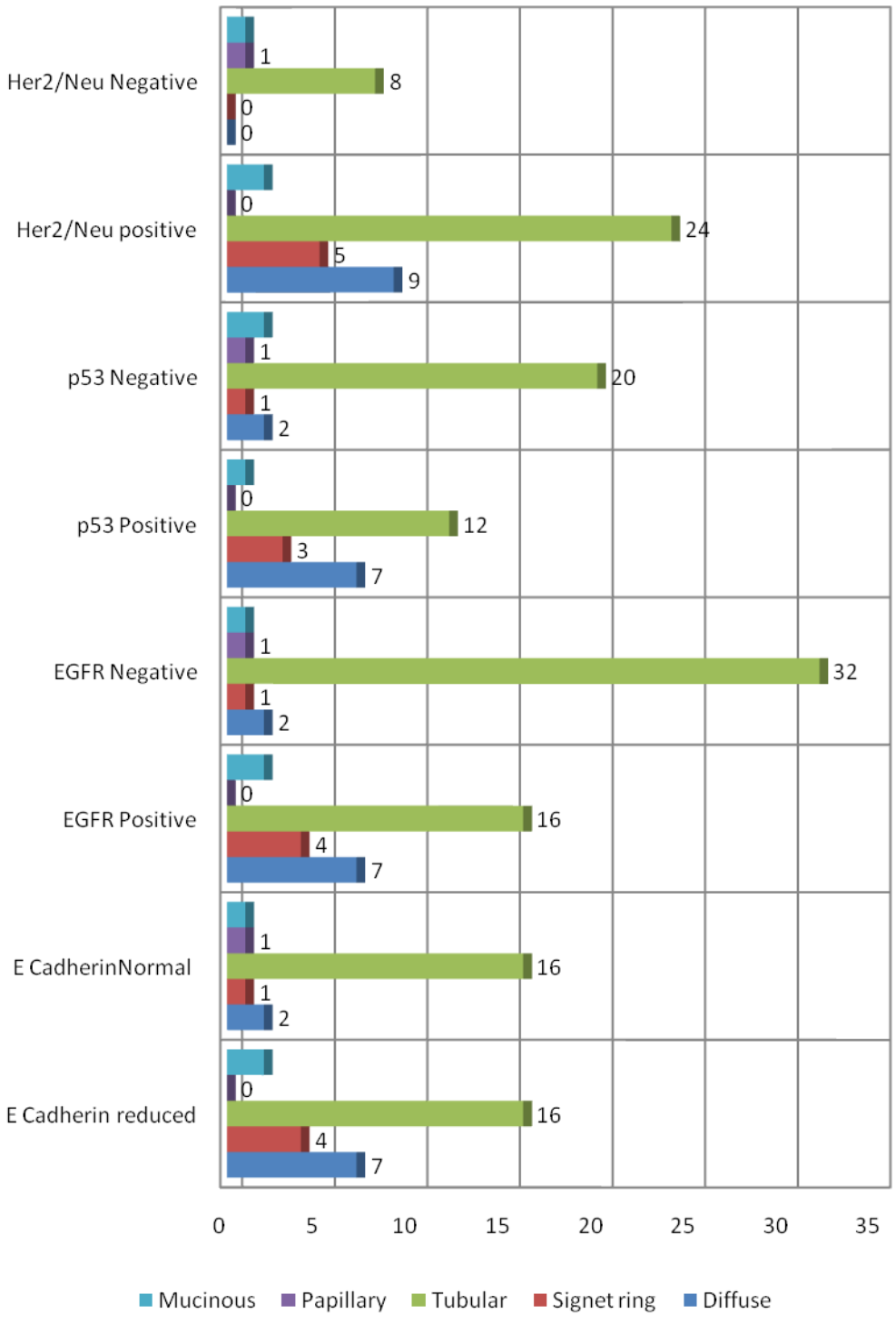


Chart 16: Histological type Vs ECadherin, EGFR, p53, Her2/Neu



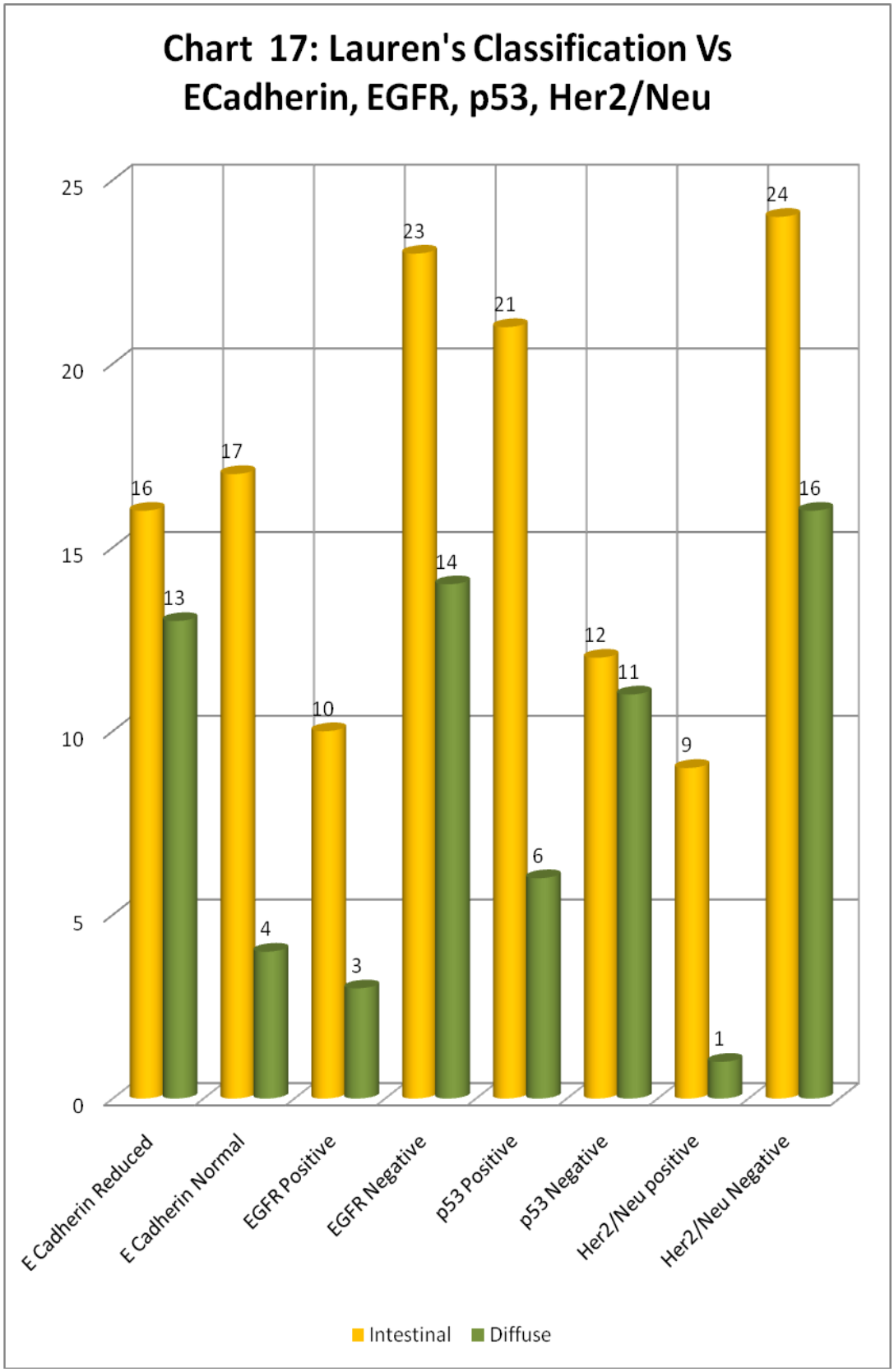
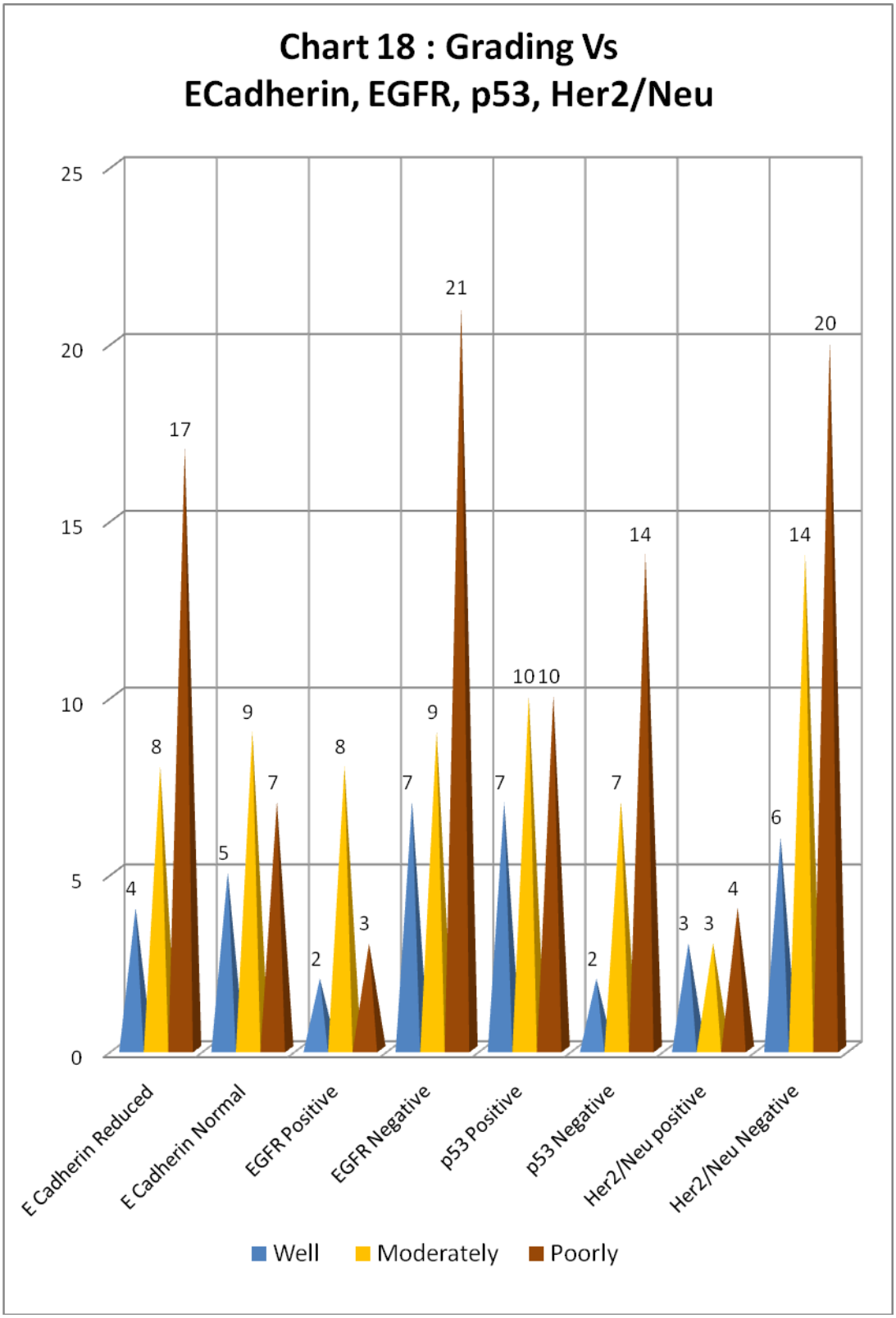


Chart 17: Lauren's Classification Vs ECadherin, EGFR, p53, Her2/Neu
25
20
15
10
5
0
16
13
17
4
10
3
23
14
21
6
12
11
9
1
24
16
E Cadherin Reduced
E Cadherin Normal
EGFR Positive
EGFR Negative
p53 Positive
p53 Negative
Her2/Neu positive
Her2/Neu Negative
Intestinal
Diffuse

Chart 18 : Grading Vs ECadherin, EGFR, p53, Her2/Neu



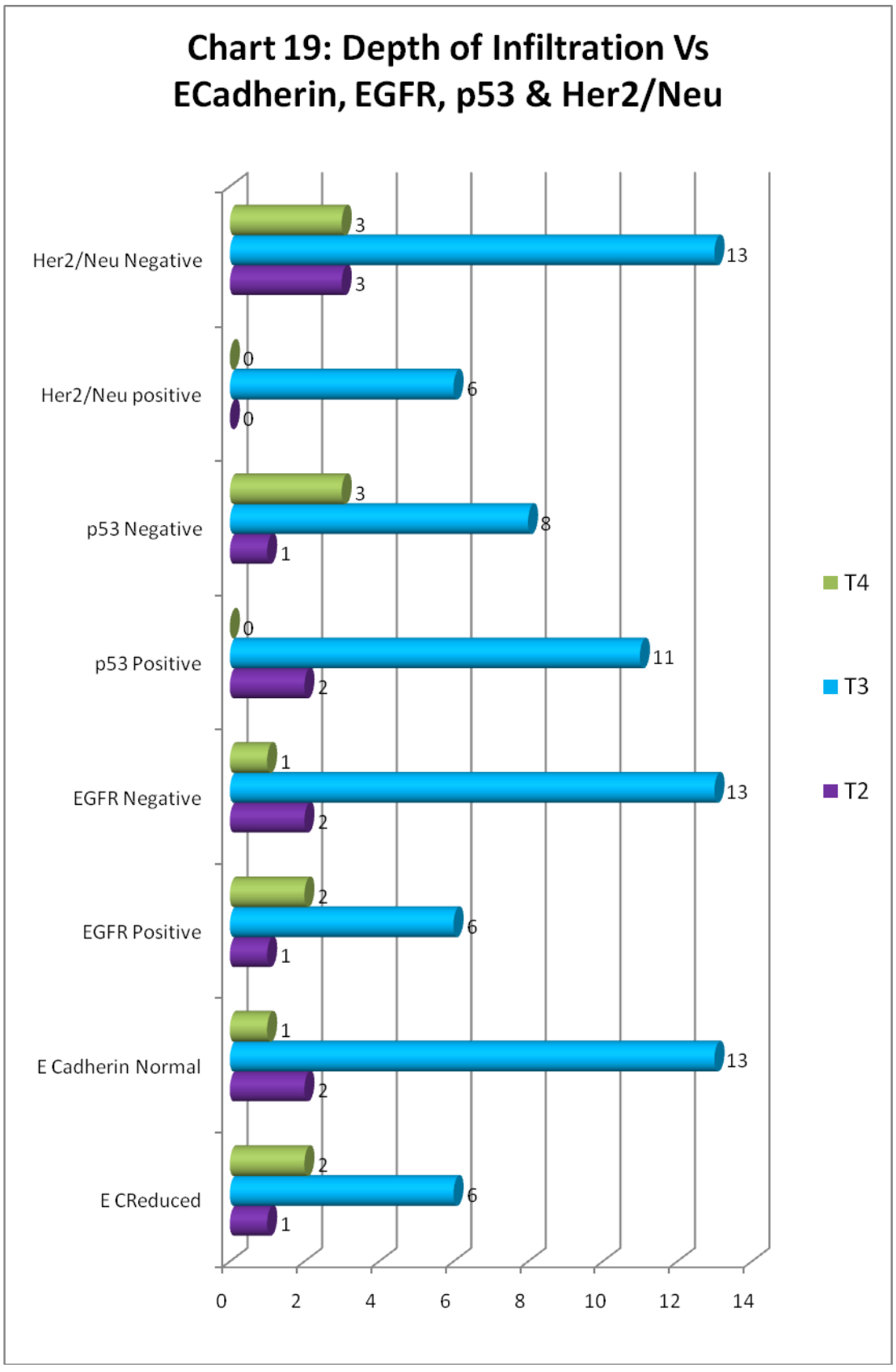


Chart 19: Depth of Infiltration Vs ECadherin, EGFR, p53 & Her2/Neu
Her2/Neu Negative
Her2/Neu positive
p53 Negative
p53 Positive
EGFR Negative
EGFR Positive
E Cadherin Normal
E CReduced
3
13
3
0
6
0
3
8
1
0
11
2
1
13
2
2
6
1
1
13
2
2
6
1
0
2
4
6
8
10
12
14
T4
T3
T2

Chart 20: Lymphatic Invasion Vs ECadherin, EGFR, p53 & Her2/Neu

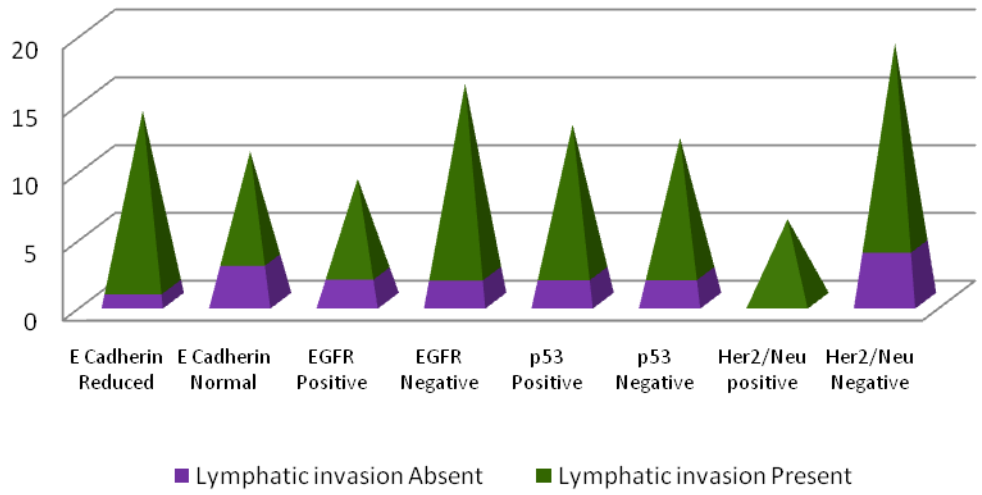


Chart 21: Vascular Invasion Vs ECadherin, EGFR, p53 & Her2/Neu

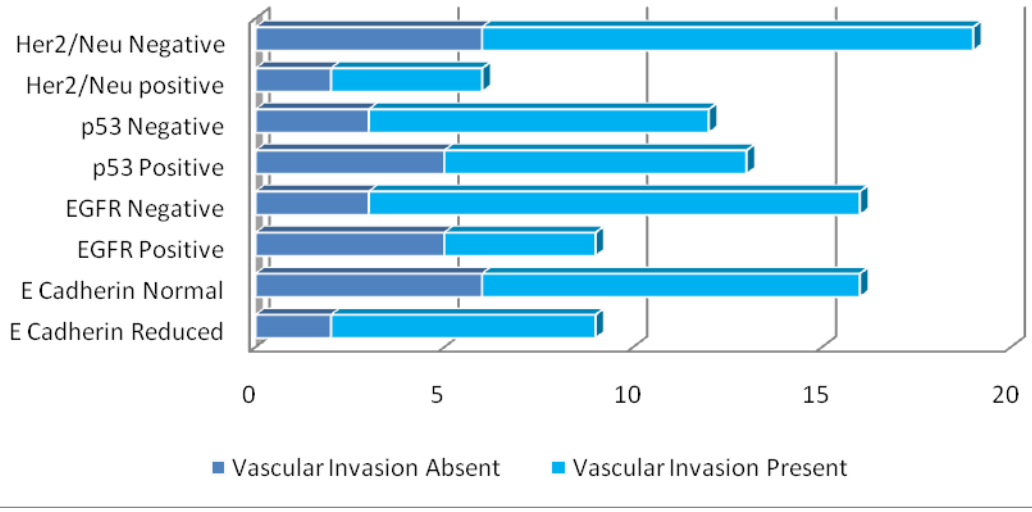


Chart 22: Perineural Invasion Vs ECadherin, EGFR, p53 & Her2/Neu

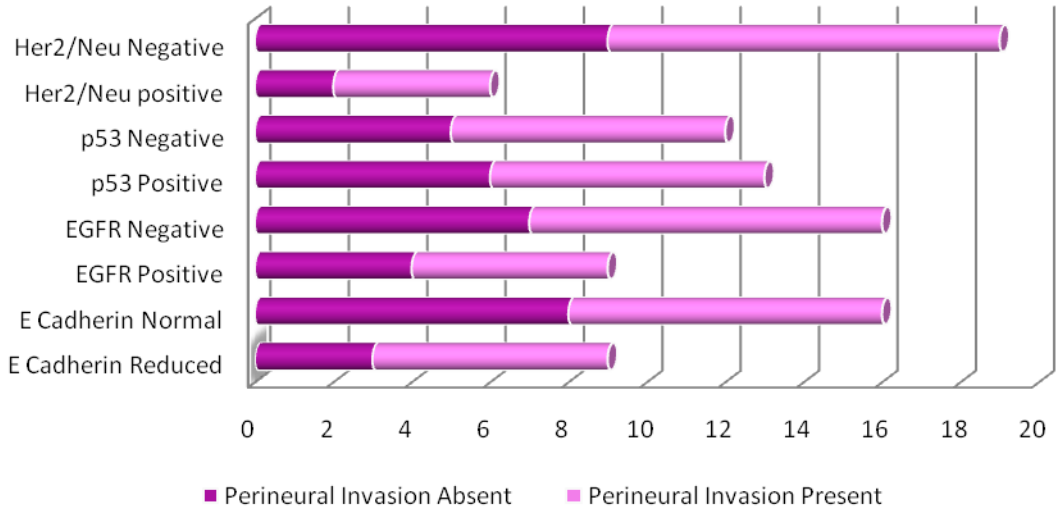
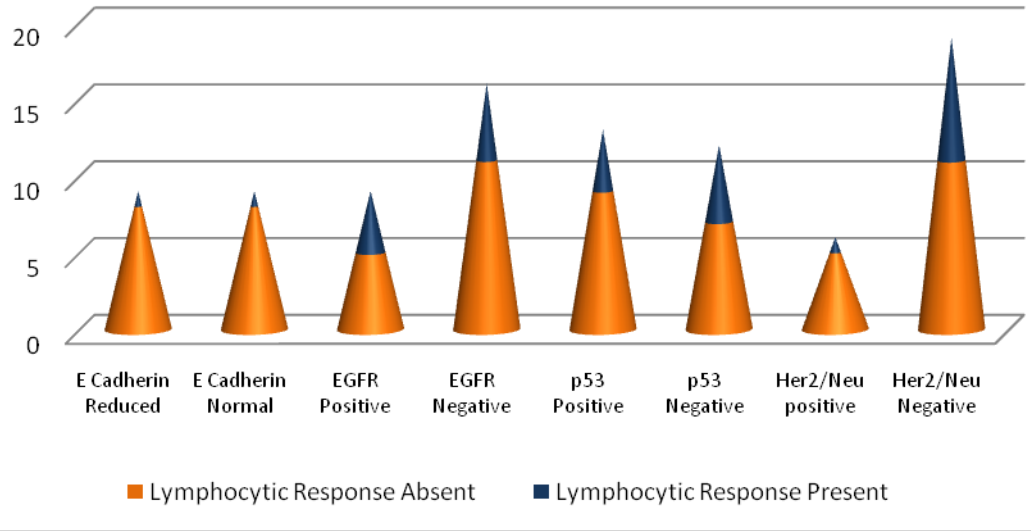


Chart 23 :Lymphocytic Response Vs ECadherin, EGFR, p53 & Her2/Neu



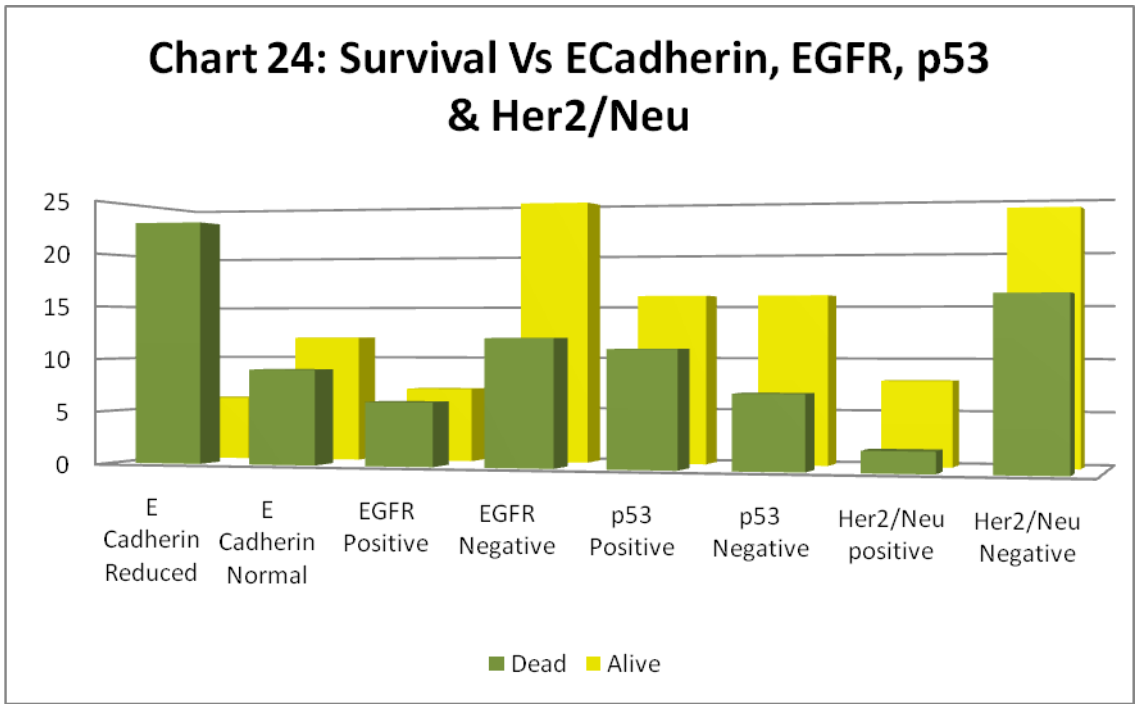


Chart 24: Survival Vs ECadherin, EGFR, p53 & Her2/Neu
25
20
15
10
5
0
E Cadherin Reduced
E Cadherin Normal
EGFR Positive
EGFR Negative
p53 Positive
p53 Negative
Her2/Neu positive
Her2/Neu Negative
Dead
Alive

DISCUSSION

Gastric cancer is the fourth most common cancer worldwide [1]. It is a disease with a high death rate (~700,000 per year) making it the second most common cause of cancer death worldwide after lung cancer [2]. More than 50% of cases present in advanced stages which are unresectable making cure impossible. The overall 5 year survival rate is 28% irrespective of the stage at which the patient presents.

Many biological markers are being studied in several parts of the world to identify their possible role in the evaluation of gastric carcinoma and thus in clinical outcome. Among them, immunohistochemical staining for cell adhesion molecule E Cadherin, cell cycle regulator p53 and growth factor receptors HER-2/Neu and EGFR have been proposed to have prognostic value.

In this study, immunohistochemical evaluation was done in 50 gastric carcinoma cases; attempt was made to correlate the expression of E Cadherin, EGFR, p53 and HER-2/Neu with various clinicopathological factors and known prognostic factors of gastric carcinoma.

In the Institute of Pathology, Madras Medical College, 338 cases of gastric adenocarcinoma were reported during the year 2011. The age of the patients ranged from 21 years to 84 years, with a mean age of 52.5 years. The mean age of incidence in males was higher than that of females (56.49 years & 50.87 years respectively) and the difference was statistically significant. The age group showing the greatest incidence of gastric carcinoma was 55 to 64 years. This is in concurrence with the study done by Zhang HK et al [(133)], who observed a mean age of 52.2 years with the range between 25 and 75 years.

In the current study, the incidence of gastric carcinoma in males and females were 72% and 28% respectively. This is in concurrence with the study by Nobuyuki Igarashi et al[(134)] who observed an incidence of 74.1% and 25.9% in males and females respectively.

The most common site of gastric carcinoma in this study was pyloroantral region (61.83%), which is similar to the study by Czyewski J et al[(135)], showing occurrence of 60% of cases in the pyloroantral region.

Table 53: Comparison of Location of Gastric Carcinoma:

Tumour Location	H R Raziee et al[10]	C Fondevila et al[122]	Czyzwski J et al[135]	Current Study
Esocardia	37%	7%	15.6%	13.91%
Body	33%	40%	20%	19.82%
Pyloroantrum	30%	51%	60%	61.83%
Pangastric	-	2%	4.4%	4.44%

Among the various histological types, tubular carcinoma was the most common type accounting for 55.92 % of cases in this study, which is almost similar to that observed by Daniela et al[136] in their study.

Table 54: Comparison of Histological Type of Gastric cancer:

Histological type	Kakeji et al[137]	Daniela et al[136]	Current study
Diffuse	-	4.9%	21.01%
Signet ring cell	3.1%	27.8%	13.02%
Tubular	89.5%	45.9%	55.91%
Papillary	2.2%	8.3%	4.44%
Mucinous	5.2%	13.1%	5.62%

On using Lauren's classification, the most common histological subtype in this study was intestinal type (60.6%).This is in concurrence

with the findings observed by Fondevila et al[122] and Gabbert et al[119] showing 62% and 63.3% respectively.

Table 55: Comparison According to Lauren's Classification:

Lauren's type	H R Raziee et al[10]	Fondevila et al[122]	Gabbert et al[119]	Current study
Intestinal	74%	62%	63.4%	60.6%
Diffuse	21%	38%	28.5%	37.5%
Indeterminate	5%	-	8.1%	1.9%

In this study poorly differentiated grade tumours were more common than other grades accounting for 48.22% of cases, which is similar to observations made by Daniela et al[136] (64%) and Fondevila et al[122] (49%) in their studies.

Table 56: Comparison of Histological Grades of Gastric Carcinoma:

Grade	H R Raziee et al[10]	Daniela et al[136]	Fondevila et al[122]	Current study
Well differentiated	54%	3.2%	4%	18.34%
Moderately differentiated	17%	32.8%	47%	33.43%
Poorly differentiated	29%	64%	49%	48.23%

In this study, a higher proportion of tumours belonged to T3 subtype (59.13%) which is similar to the observation made by Giovanni et al[139] (66%) in their study.

Table 57: Comparison of Depth of Infiltration:

Depth of Invasion	**Joo Y E et al[138]**	**Daniela et al[136]**	**Giovanni et al[139]**	**Current study**
T1	13.4%	6.5%	-	3.23%
T2	24.3%	15.9%	25%	18.28%
T3	51.2%	27.8%	66%	59.13%
T4	11.1%	49.8%	9%	19.36%

Among the resected specimens, 80.65% had lymphatic invasion and vascular invasion was seen in 59.54% of cases. This is similar to the observations obtained by Ji Yoon Choi et al[140], showing lymphatic invasion in 79.35% of cases.

In this study, 34.45% cases showed perineural infiltration and 39.48 % cases showed lymphocytic response, this was similar to the results obtained by Luo Tianhang et al[141], showing 31.7% of cases with perineural invasion.

Table 58: Comparison of E Cadherin, EGFR, p53 & HER-2/Neu with World Statistics:

	E Cadherin Reduced Expression %	**EGFR Positivity %**	**P53 Positivity %**	**HER-2/Neu Positivity %**
Current study	58	26	54	20
Gabbert et al[119]	43	-	-	-
Barbara Mayer et al[153]	92	-	-	-
Yoshihidi Shino et al[8]	32.2	-	-	-
HongKai Zhang et al[133]	44	-	-	-
Yong Ning Zhou et al[154]	46	-	-	-
Karatzas G et al[157]	67	-	-	-
Eva Lieto et al[149]	-	44	-	-
M F Filipe et al[150]	-	18	-	-
Kim M A et al[7]	-	27.4	-	-
Zhiyoung Liam et al[117]	-	42	-	-
GennaroGalizia et al[151]	-	44	-	-
Hong Suk Song et al[152]	-	25.4	-	-
Stefano Cascinu et al[89]	-	-	50	-
T Sakaguchi et al[142]	-	-	50.9	-
Kamran G et al[143]	-	-	75	-
Y E Joo et al[138]	-	-	34.4	-
N Igarashi et al[134]	-	-	58	-
N E Tzanakis et al[144]	-	-	65	-
H R Raziee et al[10]	-	-	-	26
Cathy B M et al[145]	-	-	-	23
C Ballestin et al[146]	-	-	-	13.5
S D Xie et al[147]	-	-	-	18.8
R Vergara et al[148]	-	-	-	5.6
Xiu Li Zhang et al[118]	-	-	-	18.6

Reduced expression of E Cadherin was seen in 58% cases, over expression of EGFR and HER-2/Neu was seen in 26% & 20% cases respectively and positivity for p53 was seen in 54% cases. Comparison of expression of the above mentioned markers with other literature is shown in Table 58. Various studies showed reduced expression of E Cadherin ranging from 32% to 92%, over expression of EGFR ranging from 18% to 44% , and that of HER-2/Neu ranging from 5% to 23%, and positivity for p53 ranging from 35% to 65%. These fluctuations could be due to different methodologies used for evaluation and to varying characteristics of the studied cases.

Association of E Cadherin with various clinicopathological and prognostic factors:

Helmut E Gabbert et al[119] (1996) studied 413 cases of gastric carcinoma in Germany and found significant association between E Cadherin expression and Lauren's classification, histological type and grade and no association between depth of infiltration, lymphatic invasion and vascular invasion.

Hong-Kai Zang et al[133] (2004) studied 74 cases in China and found significant association of E Cadherin expression with grade of

tumour but not with age, sex, location of tumour, size and histological type of tumour.

Yong-Ning Zhou et al[154] (2002) studied 100 cases of gastric carcinoma in China and found significant association between E Cadherin expression and gross appearance, histological grade but not with tumour size and Lauren's classification.

Yoo Y E et al[155] (2006) studied 114 cases of gastric carcinoma in Korea and found significant association of E Cadherin expression with Lauren's classification, but not with size of tumour and depth of infiltration.

Hui-Chun A Chen et al[156] (2003) studied 84 cases in Taiwan and found significant association between E Cadherin expression and tumour grade, Lauren's classification and depth of infiltration and no association with survival.

In the present study, there was a direct significant association between E Cadherin expression and survival, reduced E Cadherin expression had poor survival. Reduced expression of E Cadherin was increased in elderly age group and slight male preponderance was noted. Reduced expression of E Cadherin was seen to be increasing with increasing grade, but statistically significant association could not be ascertained.

In comparison with the above studies, this study also showed no significant association between E Cadherin expression and gross appearance, tumour size, histological type, depth of infiltration, lymphatic invasion, vascular invasion and perineural invasion.

Correlation of EGFR Expression with Various Clinicopathological and Prognostic Factors:

Hong Suk Song et al[152] (2004) studied 739 cases of gastric carcinoma in Korea and found significant association between EGFR expression and high grade of tumour, perineural invasion and no association with size of tumour, Lauren's type, depth of infiltration.

Eva Lieto et al[149] (2007) studied 88 cases in Italy and found significant association between EGFR expression and increased depth of infiltration, increasing grade and poor survival and no association between EGFR expression and other clinicopathological factors

Gennara Galizia et al[151] (2007) studied 82 cases in Greece and found a significant association between EGFR expression with gross appearance of tumour, depth of infiltration, lymphovascular invasion and poor survival and no association with size of tumour, depth of infiltration, grade of tumour and survival of patients.

M.A. Kim et al[7] (2008) studied 511 cases in Korea and observed significant association between EGFR over expression and older age of patient, increasing tumour grade, increased depth of invasion, and poor survival, but no association with other parameters like lymphatic invasion, vascular invasion, gross appearance and tumour size.

Zhiyoung Liam et al[117] (2008) studied 100 cases of gastric carcinoma and found no significant association between EGFR expression and clinicopathological and prognostic factors or with survival of the patients.

In the present study, direct significant association was found between grade of tumour and EGFR expression, moderately differentiated cases showed significant increase in over expression of EGFR. Increased frequency of EGFR over expressed cases was seen with male sex, tubular histological type and T3 level of infiltration, but statistically significant association could not be ascertained. No association was found between EGFR expression and tumour location, gross appearance, size, lymphatic invasion, vascular invasion, perineural invasion and survival.

Correlation of p53 expression with Various Clinicopathological and Prognostic Factors.

Maehara Y et al[158] (1999) studied 96 cases of gastric carcinoma in Japan, and observed statistically significant association between p53 expression and tumour size, site, lymphatic invasion, vascular invasion, depth of infiltration.

Karman G et al[143] (2004) studied 52 cases of gastric carcinoma in Iran, they were able to demonstrate significant association of p53 expression with histological type, depth of infiltration and tumour grade but no association between other factors like lymphatic invasion and vascular invasion.

Y. E. Joo et al[138] (2006) studied 119 cases in Korea and was able to demonstrate significant association between p53 expression and depth of infiltration, but not with tumour grade, histological type and survival.

N. E. Tzanakis et al[144] (2009) studied 93 cases in Greece and they were able to demonstrate statistically significant association of p53 expression with tumour size, location of tumour, depth of infiltration and survival but not with histological type and grade.

Daniela Lazar et al[138] (2010) studied 61 cases in Romania and demonstrated statistically significant association of p53 with tumour

grade, Lauren's histological type, depth of infiltration and increased survival.

In the present study, positivity for p53 showed male preponderance, increased frequency of Lauren's intestinal type, moderately and poorly differentiated tumour grades, T3 level of infiltration, but statistically significant association could not be ascertained. No association of p53 positivity was found with site of tumour, gross appearance, size, lymphovascular invasion and survival.

Correlation of HER-2/Neu Expression with Various Clinicopathological and Prognostic Factors

H R Raziee et al[10] (2007) studied 100 cases of gastric carcinoma in Iran and found a significant association of HER-2/Neu over expression with Lauren's intestinal type of tumour and well differentiated grade, and no association between age, gender, location of tumour and depth of infiltration.

Zhiyong Liam et al[117] (2008) studied 100 cases in China and they found no significant association of over expression of HER-2/Neu with any clinicopathological factors or with survival.

S. D. Xie et al[147] (2009) studied 218 cases and they were able to demonstrate a statistically significant association of HER-2/Neu over

expression with survival of the patient and were not able to demonstrate association with any other known clinicopathological and prognostic factors.

P. Vergara et al[148] (2009) studied 90 cases of gastric carcinoma and found a statistically significant association of HER-2/Neu over expression with depth of infiltration and no association with grade, histological subtype or survival.

Xie Li Zhang et al[118] (2009) studied 102 cases of gastric cancer in Korea and found a significant association between HER-2/Neu over expression and Lauren's type and depth of infiltration and no association with grade, histological type or survival.

In this study, a statistically significant association was obtained between absence of lymphocytic response and HER-2/Neu over expression. HER-2/Neu over expression showed female preponderance. Increased frequency of HER-2/Neu over expressed cases were belonging to Lauren's intestinal type, poorly differentiated grade, and T3 level of infiltration, but statistically significant association could not be ascertained. No association was found between HER-2/Neu over expression and gross appearance, histological subtype, lymphovascular invasion, perineural invasion and survival.

SUMMARY

- Among the total number of 10,357 cases received in Institute of Pathology, Madras Medical College, during 2011; 667 cases were gastric specimens, of which 338 were gastric carcinoma cases; accounting for 3.26% of cases.
- Among the 667 gastric specimens, 321 were non neoplastic, 4 were benign and 352 were malignant cases.
- Gastric carcinoma had a peak incidence in the 55 to 64 years age group. The oldest age of presentation was 84 years and the youngest age of presentation was 21 years.
- Mean age of incidence was higher in males (56.49 years) than females (50.87 years).
- Among the 338 carcinoma cases 93 were resected specimens and 245 were endoscopic biopsies.
- There was a male preponderance; 72% of the cases were males.
- The most common site of occurrence was pyloroantral region (61.83%).
- Ulceroproliferative type was the most common morphological type accounting for 58.88% of cases.

- The most common histological type was tubular carcinoma accounting for 55.91% of gastric carcinoma cases.
- According to Lauren's classification the most common subtype was intestinal type accounting for 60.6% of cases.
- Poorly differentiated histological grade was the most common grade constituting for 48.23% of gastric carcinoma cases.
- Most cases presented with T3 level of invasion, accounting for 59.13% of cases.
- Most cases (73.1%) were less than 5 cm in size.
- Lymphatic invasion was noted in 80.65% of cases.
- Vascular invasion was seen in 59.14% of cases.
- 34.41% of cases showed perineural invasion.
- Lymphocytic response was seen in 39.78% of cases.
- Reduced expression of E Cadherin was seen in 29% of cases.
- Over expression of EGFR was seen in 26% of cases.
- 54% of cases were positive for p53.
- HER-2/Neu over expression was seen in 20% of cases.
- E Cadherin showed statistically significant association with survival of the patient, reduced expression was associated with reduced survival.

- Increase in number of cases with reduced E Cadherin expression was seen with Lauren's intestinal type, poorly differentiated grade, presence of lymphatic invasion and absence of lymphocytic response.
- No association was found between E Cadherin expression and age, sex, site, gross type, size, histological type, depth of infiltration, vascular invasion and perineural invasion.
- A statistically significant association was found between EGFR expression and moderately differentiated grade of tumour.
- Increase in the number of cases with EGFR over expression was seen with tubular type of carcinoma, Lauren's intestinal type and absence of vascular invasion.
- No association was found between EGFR expression and age, sex, site, gross type, size, depth of infiltration, lymphatic invasion, perineural invasion, lymphocytic response and survival.
- Increase in frequency of cases with p53 positivity was seen with tubular type, Lauren's intestinal type, moderately and poorly differentiated grades and T3 level of infiltration.
- No association was found between p53 expression and age, sex, site, gross type, size, lymphovascular invasion, perineural invasion, lymphocytic response and survival.

- A significant association was found between HER-2/Neu over expression and absence of lymphocytic response in the adjacent foci of tumour.
- Increase in number of cases with HER-2/Neu over expression was found to be associated with female sex, Lauren's intestinal type and T3 level of infiltration.
- No association was found between HER-2/Neu expression and age, site, gross type, size, histological type, grade, lymphovascular invasion, perineural invasion and survival.

CONCLUSION

The incidence of gastric carcinoma in the year 2011 in RGGGH was 3.26%. Many patients were older than 55 years of age with male preponderance which is similar to several other studies conducted throughout the world. Females showed a younger mean age of incidence compared to males. 29% of cases showed reduced expression of E Cadherin. A significant association was found between reduced expression of E Cadherin and reduced survival. A significant association of EGFR over expression was found with moderately differentiated grade. No significant association was found between EGFR expression and survival. An increased frequency of cases with p53 positivity showed intestinal type of Lauren's classification, moderately and poorly differentiated grades and no association between p53 positivity and survival was found. All the cases which showed HER-2/Neu over expression showed T3 level of infiltration, no association with HER-2/Neu expression and survival was found.

To conclude, identifying the expression of E Cadherin, EGFR, P53 and HER-2/Neu in gastric carcinoma can help to identify patients with reduced survival and to identify eligible candidates for targeted therapy. A larger sample size and follow up for a longer period might shed more light on the role of the above markers in gastric carcinoma.

ANNEXURE – I
PROFORMA

Case number : Name :

HPE number : Age :

IP number : Sex :

Clinical history :

Risk factors, if any :

Clinical diagnosis :

Imaging :

Endoscopy :

Previous HPE report:

Nature of specimen : Total gastrectomy/Subtotal gastrectomy/Endoscopic biopsy

GROSS:

Proximal circumference : Greater curvature:

Distal circumference : Lesser curvature :

Tumour site :

Tumour size :

Tumour configuration : Depth of invasion:

Margins : Proximal : Distal :

Associated findings :

MICROSCOPY:

Histological type :

Histological grade : G1 / G2 / G4 / G4

Lauren's classification:

Depth of invasion :

Margins : Proximal : Free / Involved

Distal : Free / Involved

Lymphatic invasion : Present / Absent

Venous invasion : Present / Absent

Perineural invasion : Present / Absent

Lymphocytic response : Present / Absent

Associated findings:

IMMUNOHISTOCHEMISTRY

E Cadherin: Intensity &Percentage of cells showing staining-

EGFR: Intensity &Percentage of cells showing staining-

P53 score : Intensity &Percentage of cells showing staining-

HER-2/Neu: Intensity &Percentage of cells showing staining-

ANNEXURE - II
WHO CLASSIFICATION OF GASTRIC TUMOURS

EPITHELIAL TUMOURS

Intraepithelial neoplasia –
Adenoma

Carcinoma

Adenocarcinoma
Intestinal type
Diffuse type
Papillary adenocarcinoma
Tubular adenocarcinoma
Mucinous adenocarcinoma
Signet-ring cell carcinoma
Adenosquamous carcinoma
Squamous cell carcinoma
Small cell carcinoma
Undifferentiated carcinoma
Others
Carcinoid (well differentiated endocrine neoplasm)

NON-EPITHELIAL TUMOURS

Leiomyoma
Schwannoma
Granular cell tumour
Glomus tumour
Leiomyosarcoma
GI stromal tumour
-Benign
-Uncertain malignant potential
-Malignant
Kaposi sarcoma
Others

Malignant lymphomas
-Marginal zone B-cell lymphoma of MALT-type
-Mantle cell lymphoma
-Diffuse large B-cell lymphoma
-Others

SECONDARY TUMOURS

ANNEXURE III
TNM STAGING OF GASTRIC TUMOURS

T – Primary Tumour

TX - Primary tumour cannot be assessed
T0 - No evidence of primary tumour
Tis - Carcinoma in situ
T1 - Tumour invades lamina propria or submucosa
- T1a-Tumour invades lamina propria or muscularis mucosa
- T1b- Tumour invades submucosa

T2 - Tumour invades muscularis propria
T3 - Tumour penetrates subserosa without invasion of serosa
T4 - Tumour invades serosa or adjacent structures
- T4a- Tumour invades serosa
- T4b- Tumour invades adjacent structures

N – Regional Lymph Nodes

NX - Regional lymph nodes cannot be assessed
N0 - No regional lymph node metastasis
N1 - Metastasis in 1 to 2 regional lymph nodes
N2 - Metastasis in 3 to 6 regional lymph nodes
N3 - Metastasis in more than 7 regional lymph nodes

M – Distant Metastasis

MX - Distant metastasis cannot be assessed
M0 - No distant metastasis
M1 - Distant metastasis

STAGE GROUPING

Stage 0	Tis	N0	M0
Stage IA	T1	N0	M0
Stage IB	T1	N1	M0
	T2	N0	M0
Stage IIA	T1	N2	M0
	T2	N1	M0
	T3	N0	M0
Stage IIB	T1	N3	M0
	T2	N2	M0
	T3	N1	M0
Stage IIIA	T2	N3	M0
	T3	N2	M0
	T4a	N1	M0
Stage IIIB	T2	N3	M0
	T3	N2	M0
	T4a	N1	M0
Stage IIIC	T4a	N2	M0
	T4b	N0	M0
	T4b	N1	M0
Stage IV	Any T	Any N	M1

ANNEXURE IV
IMMUNOHISTOCHEMISTRY PROCEDURE

1. 4μ thick sections were cut from formalin fixed paraffin embedded tissue samples and transferred to gelatin-chrome alum coated slides.
2. The slides were incubated at 58ºC for overnight.
3. The sections were deparaffinized in xylene for 15 minutes x 2 changes.
4. The sections were dehydrated with absolute alcohol for 5 minutes x 2 changes.
5. The sections were washed in tap water for 10 minutes.
6. The slides were then immersed in distilled water for 5 minutes.
7. Heat induced antigen retrieval was done with microwave oven in appropriate temperature with citrate buffer for 20 to 25 minutes.
8. The slides were then cooled to room temperature and washed in running tap water for 5 minutes.
9. The slides were then rinsed in distilled water for 5 minutes.
10. Wash with appropriate wash buffer (phosphate buffer) for 5 minutes x 2 changes.
11. Apply peroxidase block over the sections for 10 minutes.
12. Wash the slides in phosphate buffer for 5 minutes x 2 changes.
13. Cover the sections with power block for 15 minutes.
14. The sections were drained (without washing) and appropriate primary antibody was applied over the sections and incubated for 1 hour.
15. The slides were washed in phosphate buffer for 5 minutes x 2 changes.
16. The slides were covered with Super Enhancer for 30 minutes.
17. The slides were washed in phosphate buffer for 5 minutes x 2 changes.
18. The slides were covered with SS Label for 30 minutes.
19. Wash in phosphate buffer for 5 minutes x 2 changes.
20. DAB substrate was prepared by diluting 1 drop of DAB chromogen to 1 ml of DAB buffer.
21. DAB substrate solution was applied on the sections for 8 minutes.
22. Wash with phosphate buffer solution for 5 minutes x 2 changes.
23. The slides were washed well in running tap water for 5 minutes.
24. The sections were counterstained with Hematoxylin stain for 2 seconds (1 dip).
25. The slides were washed in running tap water for 3 minutes.
26. The slides are air dried, cleared with xylene and mounted with DPX.

ANNEXURE V
SCORING SYSTEM FOR THE IMMUNOHISTOCHEMICAL MARKERS

E Cadherin:

3+: preserved, continuous, linear, intercellular staining (similar to that of normal gastric mucosa) or densely dotted intercellular staining in more than 60% of all tumour cells;

2+: moderately reduced linear or dotted intercellular staining in 20-60% of all tumour cells;

1+: highly reduced, predominantly finely dotted intercellular staining in less than 20% of all tumour cells;

0 : no E-Cadherin expression or very weak dotted staining in less than *5%* of all tumour cells.

3+: *Normal*; *2+,1+, 0* : *reduced expression.*

EGFR

0 : no discernible staining or background type staining;

1+: discontinuous membrane staining;

2+: complete membrane staining with moderate intensity;

3+: strong and complete plasma membrane staining.

More than 10% of the cells are required to meet the criteria, 2+ and 3+ scores are classified as over expression.

P53:

p53-negative (-): immunostaining in < 10% of the tumour nuclei

p53-positive (+): immunostaining in > 10% of the tumour nuclei

HER-2/Neu:

0 :no discernible staining or background type staining;

1+ : discontinuous membrane staining;

2+ : membrane staining with moderate intensity

3+ : strong and complete plasma membrane staining.

More than 10% of the cells are required to meet the criteria for HER2 analysis. 3+ cases are classified as over expression.

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S.No	HPE No	Age	Sex	Proc	Site	Gross	Dia	Hist type	Lauren	Grade	Depth	Margin	LI	VI	PNI	Lymp	E Cad	EGFR	p53	Her2/neu	Follow up
1	68/11	58	1	3	1	2		3	1	1											
2	155/11	70	1	3	3	1		3	1	2											
3	156/11	50	2	3	4	3		3	1	2											
4	164/11	63	1	1	3	3	4	1	2	3	2	1	Y	Y	Y	Y					
5	241/11	45	1	3	1	1		5	2	3											
6	243/11	62	1	3	4	3		3	1	2											
7	357/11	40	1	3	4	1		3	1	2											
8	364/11	55	1	3	1	3		5	3	3											
9	367/11	60	1	3	4	3		5	2	3											
10	373/11	62	1	1	4	3	7	2	2	3	3	1	Y	N	N	Y					
11	470/11	37	1	3	3	3		3	1	2											
12	484/11	60	1	3	4	2		3	1	1											
13	485/11	28	2	3	4	3		2	2	3											
14	556/11	56	2	3	4	3		3	1	3											
15	561/11	50	1	3	4	1		3	1	1											
16	563/11	40	1	3	4	2		5	2	3											
17	576/11	70	1	1	4	1	2	3	1	1	2	1	Y	Y	N	N	1	1	Y	2	1
18	617/11	45	1	3	3	1		3	1	2											
19	637/11	45	1	1	4	3	8	3	1	3	3	1	Y	Y	N	Y					
20	702/11	61	1	3	3	3		3	1	2											

S.No	HPE No	Age	Sex	Proc	Site	Gross	Dia	Hist type	Lauren	Grade	Depth	Margin	LI	VI	PNI	Lymp	E Cad	EGFR	p53	Her2/neu	Follow up
21	703/11	40	2	3	3	1		1	2	3											
22	742/11	64	1	3	4	2		3	1	1											
23	763/11	58	1	1	4	3	5	3	1	1	3	1	Y	Y	Y	N	3	3	N	3	2
24	798/11	60	1	3	1	2		3	1	2											
25	806/11	64	1	3	4	1		2	2	3											
26	992/11	60	1	3	5	4		3	1	1											
27	1051/11	84	2	3	1	3		3	1	1											
28	1053/11	65	2	3	4	3		3	3	3											
29	1126/11	54	1	3	2	1		3	1	2							2	1	Y	1	2
30	1128/11	59	1	3	2	1		1	2	3											
31	1167/11	65	1	3	4	3		3	1	3											
32	1279/11	40	2	3	5	4		3	1	3							3	0	Y	3	2
33	1306/11	49	1	1	4	3	3	3	1	1	3	1	N	Y	N	Y	3	0	Y	0	1
34	1355/11	75	1	1	4	2	3	3	1	2	3	1	Y	Y	Y	N	3	2	Y	1	1
35	1386/11	59	1	3	1	3		5	2	3											
36	1467/11	42	1	3	1	3		3	1	3							3	0	Y	3	2
37	1473/11	65	1	3	4	3		3	1	1											
38	1474/11	75	1	3	3	1		3	1	2											
39	1480/11	21	2	3	3	1		1	2	3											
40	604/11	45	2	1	4	1	8	4	1	1	3	1	Y	Y	Y	y	3	2	Y	3	2

S.No	HPE No	Age	Sex	Proc	Site	Gross	Dia	Hist type	Lauren	Grade	Depth	Margin	LI	VI	PNI	Lymp	E Cad	EGFR	p53	Her2/neu	Follow up
41	605/11	50	1	1	3	1	2	3	1	1	3	1	N	N	N	N					
42	628/11	46	2	1	4	1	4	3	1	2	4	1	Y	Y	Y	N	2	1	N	1	2
43	638/11	60	1	3	3	1		3	1	2											
44	751/11	60	1	2	4	3	11	4	1	2	4	1	Y	Y	Y	N					
45	780/11	55	1	3	4	3		1	2	3											
46	903/11	50	1	1	4	1	10	4	1	2	4	1	Y	Y	Y	N					
47	959/11	50	2	3	3	3		2	2	3											
48	1062/11	55	1	1	4	1	4	3	1	2	4	1	Y	Y	N	N					
49	1246/11	48	1	1	4	3	3	3	1	1	1	1	N	N	N	N					
50	1362/11	55	1	3	4	2	4	1	2	3							0	0	N	0	1
51	1418/11	70	1	1	4	3	3	3	1	2	3	1	Y	N	Y	N	3	1	Y	1	1
52	74/11	72	2	1	4	3	5	3	1	1	3	3	Y	N	Y	N	3	0	Y	3	2
53	101/11	60	1	1	4	3	4	3	1	2	3	1	Y	N	Y	N	2	3	Y	1	2
54	196/11	70	1	1	4	1	8	5	2	3	4	2	Y	Y	N	N					
55	237/11	40	1	3	4	3		2	2	3											
56	327/11	65	1	1	4	1	5	5	2	3	3	1	N	N	N	N					
57	352/11	38	2	1	4	3	4	3	1	2	3	1	Y	N	N	N	3	3	Y	3	1
58	522/11	62	1	1	4	3	3	3	1	2	4	1	Y	Y	N	Y					
59	1486/11	40	2	2	3	3	9	3	1	3	3	2	Y	N	N	N					
60	1536/11	51	2	3	2	3		1	2	3											

S.No	HPE No	Age	Sex	Proc	Site	Gross	Dia	Hist type	Lauren	Grade	Depth	Margin	LI	VI	PNI	Lymp	E Cad	EGFR	p53	Her2/neu	Follow up
61	1603/11	52	1	3	1	1		3	1	2											
62	1604/11	62	2	3	4	3		4	1	2											
63	1607/11	65	1	3	4	3		3	1	2											
64	1608/11	56	1	3	4	3		4	1	1											
65	1613/11	63	1	3	4	3		4	2	3											
66	1617/11	35	2	3	4	3		3	1	2							2	0	Y	0	2
67	1682/11	47	1	3	3	3		2	2	3											
68	1684/11	76	1	3	3	3		2	2	3											
69	1712/11	49	2	3	4	3		3	1	2							3	0	Y	0	2
70	1759/11	70	1	3	4	3		3	1	2											
71	1761/11	45	1	3	3	3		3	1	2											
72	1871/11	35	1	3	4	3		3	1	2											
73	1950/11	60	1	3	4	3		3	1	2											
74	2017/11	65	1	3	3	1		2	2	3											
75	2048/11	62	2	1	4	3	3	3	1	2	2	1	Y	Y	N	Y					
76	2071/11	65	1	3	4	3		3	1	1											
77	2190/11	44	2	3	4	1		2	2	3											
78	2215/11	47	1	3	4	3		3	1	2							2	2	Y	1	2
79	2217/11	57	1	3	1	2		1	2	3											
80	2218/11	50	2	3	3	1		2	2	3											

S.No	HPE No	Age	Sex	Proc	Site	Gross	Dia	Hist type	Lauren	Grade	Depth	Margin	LI	VI	PNI	Lymp	E Cad	EGFR	p53	Her2/neu	Follow up
81	2259/11	29	2	1	4	1	4	1	2	3	3	1	Y	Y	N	N					
82	2339/11	70	2	3	4	3		1	2	3											
83	2382/11	70	1	3	3	3		3	1	1											
84	2426/11	55	2	3	4	3		3	1	1											
85	2512/11	55	1	3	3	1		2	2	3											
86	2518/11	50	2	3	4	3		4	1	2											
87	2591/11	55	1	3	3	3		3	1	2											
88	2592/11	50	2	3	4	3		3	1	2											
89	2593/11	47	1	3	3	1		3	1	2											
90	2662/11	52	1	3	4	3		5	2	3											
91	2732/11	42	1	3	3	3		1	2	3											
92	2862/11	55	1	3	4	3		3	1	1											
93	2865/11	40	2	3	3	3		5	2	3											
94	2959/11	70	1	3	4	3		2	2	3											
95	2965/11	50	1	3	3	3		2	2	3											
96	2967/11	60	2	3	5	5		2	2	3							0	0	Y	2	1
97	3073/11	53	1	3	4	3		3	1	1							2	0	N	1	2
98	3143/11	49	1	3	5	1		1	2	3											
99	3154/11	28	1	3	4	3		1	2	3											
100	3254/11	71	1	3	3	3		1	2	3											

S.No	HPE No	Age	Sex	Proc	Site	Gross	Dia	Hist type	Lauren	Grade	Depth	Margin	LI	VI	PNI	Lymp	E Cad	EGFR	p53	Her2/neu	Follow up
101	3429/11	46	2	3	4	3		1	2	3											
102	3494/11	50	1	1	4	3	4	3	1	1	4	1	Y	Y	Y	N	N				
103	3502/11	65	1	3	4	3		3	1	1											
104	3505/11	72	1	3	4	3		3	1	2											
105	3506/11	40	1	3	4	3		1	2	3											
106	3747/11	58	1	3	3	1		1	2	3											
107	3767/11	50	1	3	1	3		2	1	2											
108	3768/11	55	1	3	1	3		3	1	1											
109	3771/11	55	1	3	4	3		3	1	1											
110	3774/11	50	2	3	2	1		1	2	3											
111	3813/11	54	1	1	4	3	2	3	1	3	4	1	Y	Y	N	Y					
112	3849/11	32	1	3	3	3		2	1	2											
113	3874/11	45	2	3	3	2		3	1	2											
114	3902/11	35	1	3	3	3		1	2	3											
115	3903/11	42	1	3	4	3		1	2	3											
116	3905/11	45	1	3	3	3		2	2	3											
117	3928/11	58	1	3	2	3		2	2	3											
118	1658/11	52	1	2	2	3	3	3	1	3	3	1	Y	Y	N	N	3	0	Y	3	2
119	1691/11	46	2	1	4	1	4	3	1	2	4	1	Y	Y	Y	N					
120	1842/11	27	2	1	4	2	5	3	1	3	4	1	Y	Y	N	Y					

S.No	HPE No	Age	Sex	Proc	Site	Gross	Dia	Hist type	Lauren	Grade	Depth	Margin	LI	VI	PNI	Lymp	E Cad	EGFR	p53	Her2/neu	Follow up
121	1861/11	57	1	1	4	3	5	3	1	3	3	1	Y	Y	Y	N	0	0	N	2	2
122	2468/11	60	1	1	4	1	4	3	1	2	4	1	Y	Y	N	N					
123	3080/11	35	2	3	4	3		3	1	1											
124	3279/11	50	2	2	1	3	5	1	2	3	4	1	Y	Y	N	N	3	0	N	2	1
125	3426/11	42	2	3	2	3		1	2	3											
126	3428/11	48	1	3	4	3		1	2	3											
127	3430/11	60	1	3	4	2		3	1	2							0	0	N	1	2
128	3432/11	32	2	3	3	3		3	1	2											
129	3457/11	54	1	1	4	3	7	1	2	3	3	1	Y	Y	Y	N					
130	3459/11	56	1	3	3	1		2	1	2											
131	3538/11	62	1	3	4	1		3	1	1											
132	3585/11	71	1	2	3	3	3	3	1	2	2	1	Y	Y	N	N					
133	3623/11	60	1	1	4	3	3	1	2	3	4	1	Y	Y	Y	N					
134	3670/11	62	1	1	4	1	4	3	1	2	3	1	Y	N	N	N					
135	3914/11	50	1	2	3	3	9	5	2	3	3	1	Y	Y	Y	N	0	0	N	3	1
136	2972/11	60	2	2	5	5	12	1	2	3	4	1	Y	Y	Y	N	0	0	N	0	2
137	2836/11	50	1	1	4	1	3	3	1	1	3	1	Y	Y	N	Y	3	0	Y	0	1
138	3968/11	46	1	3	4	3		2	2	3											
139	3970/11	28	1	3	4	3		1	2	3											
140	3977/11	32	1	3	4	3		2	2	3							0	0	Y	0	1

S.No	HPE No	Age	Sex	Proc	Site	Gross	Dia	Hist type	Lauren	Grade	Depth	Margin	LI	VI	PNI	Lymp	E Cad	EGFR	p53	Her2/neu	Follow up
141	4039/11	56	1	3	4	2		3	1	2											
142	4040/11	40	2	3	4	3		1	2	3											
143	4042/11	60	1	3	4	3		3	1	2											
144	4140/11	57	1	3	4	1		3	1	1											
145	4337/11	60	2	3	4	3		2	2	3							0	3	N	1	2
146	4359/11	70	1	3	3	1		4	1	1											
147	4389/11	58	1	3	4	1		3	1	1											
148	4425/11	35	1	3	4	3		3	1	1							2	0	Y	1	2
149	4486/11	40	1	3	4	3		1	2	3											
150	994/11	64	1	3	2	3		3	1	2							2	3	N	0	2
151	487/11	60	1	3	5	4		1	2	3											
152	4086/11	39	2	3	4	1		3	1	2							3	2	N	3	2
153	4090/11	60	2	3	4	3		3	1	2							3	0	Y	3	2
154	4144/11	62	1	3	3	3		3	1	2											
155	4214/11	47	1	3	4	3		3	1	2											
156	4312/11	70	2	3	2	1		1	2	3							0	0	Y	0	2
157	4589/11	75	1	3	4	3		3	1	2											
158	4685/11	39	1	3	4	1		3	1	3											
159	4804/11	60	1	1	4	3	3	3	1	1	3	1	Y	Y	N	N					
160	4821/11	41	1	3	1	2		1	2	3											

S.No	HPE No	Age	Sex	Proc	Site	Gross	Dia	Hist type	Lauren	Grade	Depth	Margin	LI	VI	PNI	Lymp	E Cad	EGFR	p53	Her2/neu	Follow up
161	4822/11	56	1	3	3	1		1	2	3											
162	4865/11	46	2	3	1	3		1	2	3											
163	4850/11	48	1	1	3	1	4	1	2	3	3	1	Y	Y	N	Y					
164	4890/11	75	1	3	4	2		3	1	1											
165	4940/11	80	1	3	4	3		2	2	3											
166	4941/11	68	1	3	4	3		3	1	2											
167	4975/11	60	1	3	4	3		2	2	3											
168	4978/11	40	2	3	4	3		2	2	3											
169	4979/11	47	1	3	3	2		3	1	3											
170	5019/11	40	1	1	4	1	4	3	1	2	3	1	Y	Y	N	Y					
171	5065/11	36	2	3	4	3		3	1	2											
172	5093/11	60	2	1	4	3	7	5	2	3	3	1	Y	Y	Y	N	3	0	Y	1	1
173	5121/11	70	1	3	3	2		3	1	1											
174	5144/11	67	1	3	4	3		3	1	1											
175	5195/11	53	1	3	1	3		3	1	3											
176	5198/11	70	1	3	4	3		1	2	3											
177	5202/11	58	1	3	1	2		1	2	3											
178	5222/11	70	2	3	4	2		2	2	3											
179	5266/11	50	1	3	3	1		1	2	3											
180	5337/11	50	1	3	4	3		2	2	3											

S.No	HPE No	Age	Sex	Proc	Site	Gross	Dia	Hist type	Lauren	Grade	Depth	Margin	LI	VI	PNI	Lymp	E Cad	EGFR	p53	Her2/neu	Follow up
181	5384/11	62	1	1	4	2	5	2	2	3	3	1	Y	Y	N	Y	3	2	N	0	1
182	5396/11	54	1	3	4	2		3	1	2											
183	5397/11	54	1	3	4	2		3	1	2											
184	5398/11	50	1	3	1	3		1	2	3							2	0	N	0	2
185	5482/11	52	1	3	4	3		1	2	3											
186	5484/11	52	1	3	4	3		2	2	3											
187	5485/11	68	1	3	3	3		3	1	2											
188	5497/11	67	1	1	4	1	2	3	1	2	4	1	Y	Y	N	Y					
189	5520/11	60	1	3	4	2		3	1	2											
190	5571/11	65	1	3	4	3		3	1	1											
191	5628/11	68	1	1	4	3	5	3	1	2	3	1	Y	N	N	Y					
192	5638/11	64	1	3	1	3		3	1	1											
193	5650/11	23	2	3	4	2		2	2	3											
194	5753/11	53	1	3	4	2		3	1	1											
195	5823/11	52	1	3	4	3		3	1	2											
196	5839/11	43	1	3	3	1		1	2	3											
197	5890/11	52	1	3	3	1		1	2	3											
198	4352/11	45	1	1	4	3	3	1	2	3	4	1	Y	Y	N	N					
199	4385/11	60	1	3	4	3		1	2	3											
200	4533/11	45	1	2	3	1	4	3	1	1	4	1	Y	Y	Y	N					

S.No	HPE No	Age	Sex	Proc	Site	Gross	Dia	Hist type	Lauren	Grade	Depth	Margin	LI	VI	PNI	Lymp	E Cad	EGFR	p53	Her2/neu	Follow up
201	4545/11	55	1	1	3	3	5	3	1	2	4	1	Y	Y	Y	N					
202	4675/11	55	1	1	4	1	5	5	2	3	4	1	Y	N	N	Y					
203	4701/11	70	2	1	4	1	9	1	2	3	2	1	Y	Y	N	N					
204	5016/11	39	1	1	4	1	4	3	1	2	4	3	Y	Y	Y	N					
205	5064/11	60	1	3	4	3		1	2	3											
206	5273/11	47	2	1	4	3	2	5	2	3	4	1	Y	N	Y	N					
207	5295/11	46	1	1	4	3	4	3	1	1	1	1	N	N	N	N					
208	5631/11	67	1	3	4	3		3	1	3							0	0	N	0	2
209	5885/11	52	1	1	4	3	5	5	2	3	2	1	N	N	N	N	2	2	Y	1	1
210	6197/11	48	1	1	4	1	3	3	1	2	3	1	N	N	Y	Y					
211	6585/11	51	1	3	4	1		1	2	3											
212	6696/11	50	1	3	1	1		1	2	3											
213	7061/11	59	1	3	4	3		4	1	1											
214	7183/11	60	2	2	3	3	6	3	1	1	4	1	Y	Y	Y	N					
215	7599/11	58	1	1	4	3	11	3	1	2	4	3	Y	N	N	N					
216	5978/11	75	1	3	4	3		3	1	2											
217	5991/11	65	1	1	4	3	7	3	1	2	4	1	N	N	Y	Y	3	2	N	0	1
218	6033/11	44	1	3	1	1		3	1	1											
219	6036/11	80	1	3	4	3		1	2	3											
220	6037/11	34	1	3	4	1		1	2	3											

S.No	HPE No	Age	Sex	Proc	Site	Gross	Dia	Hist type	Lauren	Grade	Depth	Margin	LI	VI	PNI	Lymp	E Cad	EGFR	p53	Her2/neu	Follow up
221	6038/11	70	1	3	4	3		3	1	3											
222	6060/11	61	1	1	4	1	4	1	2	3	3	1	Y	Y	N	N	2	0	N	1	2
223	6140/11	45	2	3	5	4		2	2	3							0	0	N	1	2
224	6144/11	50	2	3	5	5		2	2	3											
225	6185/11	65	1	3	3	3		3	1	1											
226	6230/11	45	2	3	1	3		1	2	3											
227	6270/11	58	1	3	5	5		3	1	1											
228	6405/11	69	1	3	5	3		3	1	1											
229	6411/11	75	1	1	4	2	4	3	1	2	2	1	N	N	N	Y	3	1	N	1	1
230	6454/11	60	1	3	4	3		3	1	1											
231	6568/11	64	1	3	1	3		1	2	3											
232	6599/11	53	1	1	4	2	3	3	1	1	1	1	N	N	N	Y					
233	6733/11	43	1	3	4	3		1	2	3											
234	6818/11	40	2	2	1	3	5	4	1	1	2	1	Y	Y	N	N					
235	6991/11	52	1	3	4	3		3	1	2											
236	6992/11	60	1	3	4	3		1	2	3											
237	6994/11	28	2	3	4	1		2	2	3											
238	7033/11	72	1	3	4	3		1	2	3											
239	7170/11	60	1	2	5	5	12	1	2	3	4	1	Y	Y	Y	N					
240	7224/11	50	2	2	5	5	15	3	1	2	2	1	Y	N	N	N					

S.No	HPE No	Age	Sex	Proc	Site	Gross	Dia	Hist type	Lauren	Grade	Depth	Margin	LI	VI	PNI	Lymp	E Cad	EGFR	p53	Her2/neu	Follow up
241	7279/11	50	2	3	3	3		1	2	3											
242	7281/11	78	1	3	4	3		3	1	2											
243	7282/11	38	2	3	4	1		3	1	2											
244	7316/11	58	1	3	3	3		5	2	3											
245	7330/11	34	1	1	4	1	2	1	2	3	3	1	N	N	N	Y					
246	7427/11	56	2	3	1	3		2	2	3											
247	7433/11	57	1	3	3	3		3	1	2											
248	7459/11	51	1	3	4	3		3	1	2											
249	7563/11	65	1	3	1	3		3	1	2											
250	7564/11	80	2	3	4	3		3	1	3											
251	7566/11	54	1	3	4	3		3	1	3											
252	7619/11	78	1	2	4	3	3	3	1	1	3	1	Y	Y	N	Y					
253	7658/11	45	1	3	1	3		2	2	3											
254	7662/11	59	1	3	1	3		4	1	1											
255	7725/11	31	2	1	4	1	3	3	1	2	3	1	Y	N	N	Y					
256	7850/11	62	1	3	4	3		3	1	1											
257	7851/11	64	1	3	4	3		1	2	3											
258	7854/11	42	1	3	4	3		1	2	3							0	0	N	1	2
259	7898/11	70	1	3	4	3		3	1	3											
260	7965/11	60	1	1	4	3	5	3	1	2	4	1	Y	N	N	Y	3	3	N	0	1

S.No	HPE No	Age	Sex	Proc	Site	Gross	Dia	Hist type	Lauren	Grade	Depth	Margin	LI	VI	PNI	Lymp	E Cad	EGFR	p53	Her2/neu	Follow up
261	7978/11	59	1	3	3	1		3	1	2											
262	7996/11	60	2	3	2	3		3	1	2							0	0	Y	1	2
263	7997/11	65	2	3	4	3		3	1	3							1	0	N	1	2
264	8004/11	70	1	3	4	3		3	1	2											
265	8005/11	51	2	3	1	3		3	1	1							1	0	y	0	1
266	8037/11	70	1	1	4	3	4	3	1	2	2	1	Y	N	N	Y					
267	8189/11	55	2	3	4	3		3	1	2											
268	8225/11	60	1	2	5	5	15	3	1	2	3	4	Y	Y	Y	N					
269	8242/11	65	2	3	5	5		1	2	3											
270	8245/11	78	2	3	4	3		3	1	3											
271	8270/11	65	2	3	4	3		3	1	3							2	0	Y	0	2
272	8271/11	60	2	3	4	3		2	2	3											
273	8272/11	50	1	3	3	3		1	2	3											
274	8288/11	40	1	3	4	3		3	1	2											
275	8318/11	58	2	1	4	3	2	3	1	2	3	1	Y	N	N	Y					
276	8356/11	70	1	1	4	2	5	5	2	3	3	1	Y	Y	N	Y					
277	8490/11	55	1	1	4	2	4	3	1	2	2	1	Y	N	N	N					
278	8528/11	55	2	1	4	3	3	3	1	2	3	1	Y	N	N	Y					
279	8558/11	55	1	3	3	2		2	2	3											
280	8329/11	53	2	3	3	4		3	1	1											

S.No	HPE No	Age	Sex	Proc	Site	Gross	Dia	Hist type	Lauren	Grade	Depth	Margin	LI	VI	PNI	Lymp	E Cad	EGFR	p53	Her2/neu	Follow up
281	8332/11	75	2	1	4	1	3	3	1	3	2	1	N	N	N	N					
282	8381/11	60	2	3	4	3		1	2	3											
283	8500/11	60	2	1	4	1	6	1	2	3	3	1	Y	N	N	Y					
284	8603/11	70	1	3	4	1		2	2	3											
285	8699/11	64	1	3	1	1		3	1	2											
286	9015/11	42	2	3	4	1		2	2	3											
287	9033/11	70	2	3	1	3		3	1	3											
288	9060/11	65	1	3	4	3		3	1	3											
289	9138/11	47	2	3	3	3		3	1	3											
290	9331/11	40	1	3	4	1		5	2	3											
291	9341/11	52	1	3	3	3		2	2	3											
292	9394/11	65	1	3	3	3		3	1	2											
293	9426/11	60	1	3	4	3		3	1	1											
294	9510/11	52	2	1	4	1	2	3	1	2	3	1	N	N	N	Y					
295	9604/11	60	1	2	4	1	6	3	1	3	3	1	Y	Y	Y	N					
296	9626/11	64	1	3	3	3		3	1	2											
297	9687/11	60	1	2	1	3	8	3	1	2	2	1	N	N	N	Y					
298	9729/11	35	1	2	5	5	15	1	2	3	3	2	Y	Y	Y	Y	3	1	Y	0	1
299	9753/11	32	1	3	4	1		3	1	2											
300	9761/11	55	1	3	3	1		4	1	2											

S.No	HPE No	Age	Sex	Proc	Site	Gross	Dia	Hist type	Lauren	Grade	Depth	Margin	LI	VI	PNI	Lymp	E Cad	EGFR	p53	Her2/neu	Follow up
301	9785/11	45	2	3	4	1		4	1	1											
302	9787/11	50	1	2	3	1	2	3	1	1	2	1	N	N	N	N					
303	9885/11	29	2	3	2	2		1	2	3											
304	9886/11	22	2	3	4	3		3	1	1											
305	9890/11	70	2	3	4	2		4	1	2											
306	9918/11	63	1	3	3	1		3	1	2											
307	9941/11	68	2	3	4	3		3	1	2											
308	9942/11	55	1	3	4	2		3	1	1											
309	9994/11	55	1	3	1	1		3	1	2											
310	10044/11	85	1	3	4	3		3	1	3											
311	10060/11	55	1	3	3	1		3	1	2											
312	10094/11	60	1	3	4	1		3	1	2											
313	10096/11	73	1	3	4	3		3	1	1											
314	10148/11	64	1	2	2	3	5	1	2	3	4	1	Y	Y	N	Y					
315	10197/11	73	1	3	4	3		3	1	3											
316	10198/11	39	1	3	4	3		2	2	3											
317	10212/11	75	1	3	4	1		3	1	2											
318	10214/11	55	2	3	1	3		2	2	3											
319	10242/11	74	1	3	4	1		3	1	3											
320	10284/11	48	1	3	4	3		3	1	2											

S.No	HPE No	Age	Sex	Proc	Site	Gross	Dia	Hist type	Lauren	Grade	Depth	Margin	LI	VI	PNI	Lymp	E Cad	EGFR	p53	Her2/neu	Follow up
312	10306/11	62	1	3	3	1		4	1	1											
322	10343/11	41	1	3	4	3		3	1	3											
323	10351/11	61	1	3	3	2		5	2	3											
324	10352/11	67	2	3	4	1		3	1	2											
325	7776/11	52	2	3	4	3		3	1	2											
326	8023/11	54	1	1	4	1	6	3	1	2	3	1	Y	Y	N	N					
327	8100/11	54	2	2	4	2	2	3	1	2	2	1	N	N	N	N					
328	8197/11	40	2	3	1	3		2	2	3											
329	8764/11	55	1	1	4	1	9	5	2	3	4	1	Y	N	N	N					
330	8896/11	37	2	1	4	3	5	1	2	3	4	1	Y	Y	Y	Y	1	0	N	0	1
331	8897/11	53	1	1	4	3	5	3	1	2	4	1	N	N	N	Y					
332	9010/11	50	1	1	3	2	2	3	1	1	2	1	N	N	N	Y					
333	9212/11	56	1	1	4	1	5	3	1	2	4	1	Y	Y	Y	N					
334	9537/11	52	1	1	4	1	6	2	2	3	2	1	Y	Y	Y	N					
335	9632/11	21	1	3	4	1		3	1	2											
336	9755/11	70	1	2	2	3	8	3	1	2	4	1	Y	Y	N	Y					
337	9812/11	68	2	1	4	3	3	1	2	3	3	1	Y	N	N	N					
338	10147/11	55	2	3	2	1		3	1	2											

KEY TO MASTER CHART:

Age:

-Entered in Years

Sex:

1- Male, 2- Female

Proc (Procedure done):

Subtotal Gastrectomy, 2- Total gastrectomy, 3- Endoscopic Biopsy

Site:

1-EsoCardia, 2-Fundus, 3-Body, 4-Pyroloantrum, 5- Pangastric

Gross:

1-Ulcerative, 2-Nodular, 3-Ulceroproliferative, 4-Linitis plastica

Dia (Maximum dimension of Tumour)

-Entered in centimetres

Hist (Histological subtype)

1- Diffuse, 2- Signet ring cell type, 3- Tubular, 4- Papillary, 5- Mucinous

Lauren (Lauren's classification)

1-Intestinal type, 2-Diffuse type, 3-Indeterminate

Grade

1-Well differentiated, 2-moderately differentiated, 3- poorly differentiated

Depth (Depth of infiltration)

1-T1, 2-T2, 3-T3, 4-T4

Margin (Surgical resected Margin):

1-Both margins free, 2-proximal margin involved, 3-distal margin involved, 4- Both margins involved

LI (Lymphatic invasion):

Y – Present, N- Absent

VI (Vascular Invasion):

Y – Present, N- Absent

PNI (Perineural Invasion):

Y – Present, N- Absent

Lymp (Lymphocytic response):

Y – Present, N- Absent

E Cad (E Cadherin):

0 - 0, 1-1+ ,2-2+, 3-3+

EGFR:

0 - 0, 1-1+ ,2-2+, 3-3+

P53:

y- Positive, N-Negative

HER-2/Neu:

0 - 0, 1-1+ ,2-2+, 3-3+

Follow up:

1- Alive, 2- Dead

ABSTRACT

AIM:

The variable prognosis of gastric cancer within a pathological stage necessitates the identification of subgroups of patients with a more aggressive disease. The role of E Cadherin, EGFR, p53 and HER-2/Neu expression in gastric carcinoma is far from being fully established. The aim of the present study was to identify the incidence and distribution of gastric carcinoma in patients admitted in the Government General Hospital, Chennai in the year 2011 and to evaluate the expression of E Cadherin, EGFR, p53 and HER-2/Neu in gastric cancer and correlate the findings with several clinico-pathological features, prognosis and survival.

MATERIALS AND METHODS:

Formalin-fixed paraffin-embedded tissue samples from 50 cases (25 endoscopic biopsies and 25 gastric resection specimens) of gastric carcinoma in the year 2011 were studied by immunohistochemistry, using monoclonal antibodies to E Cadherin, EGFR, p53 and HER-2/Neu. The results were correlated with clinico-pathological features and survival.

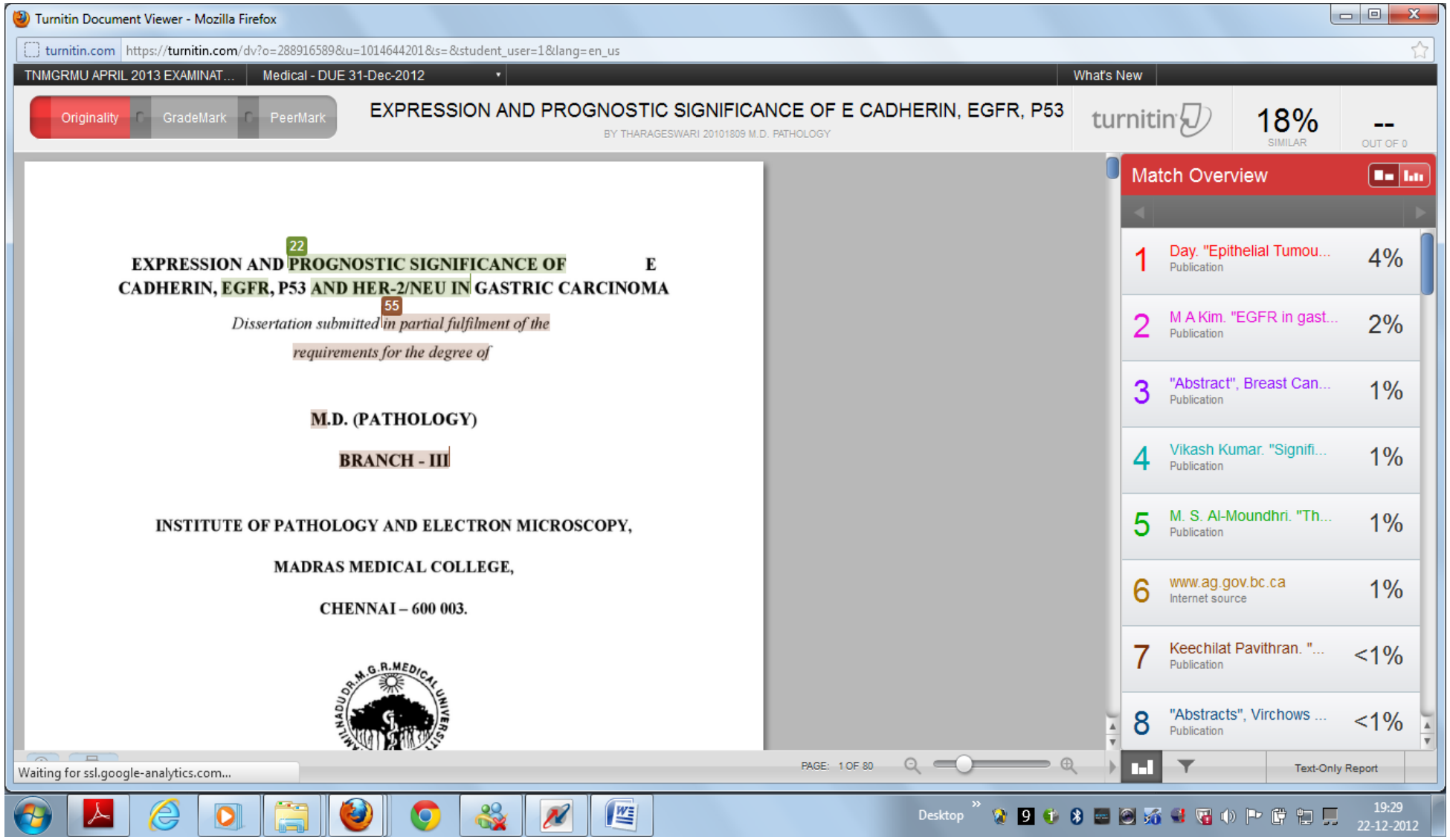
RESULTS:

Reduced expression of E Cadherin was significantly associated with reduced survival. Increase in number of cases with reduced E Cadherin expression was seen

with Lauren's intestinal type, poorly differentiated grade, presence of lymphatic invasion and absence of lymphocytic response. A statistically significant association was found between EGFR expression and moderately differentiated grade of tumour. Increase in the number of cases with EGFR over expression was seen with tubular type of carcinoma, Lauren's intestinal type and absence of vascular invasion. Increase in frequency of cases with p53 positivity was seen with tubular type, Lauren's intestinal type, moderately and poorly differentiated grades and T3 level of infiltration. A significant association was found between HER-2/Neu over expression and absence of lymphocytic response in the adjacent foci of tumour. Increase in number of cases with HER-2/Neu over expression was found to be associated with female sex, Lauren's intestinal type and T3 level of infiltration.

CONCLUSION:

The role played by cell proliferation in the growth and aggressiveness of gastric tumours is complex and still not clarified. Study of the expression of E Cadherin, EGFR, p53 and HER-2/Neu in gastric carcinoma could be useful as independent prognostic markers in identification of patients at high risk of recurrence and poor survival. Follow up of these patients for 5 more years could throw more light on the role of the above mentioned markers as long term prognostic indicators.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